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Abstract Book

Contents

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Sponsors

Oral Communications
Monday, 29 July 2024

Session: Presidential Symposium 1

Single nucleus RNA-sequencing reveals transcriptional

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As relationships mature, partners share common goals, improve their ability to work together, and experience coordinated emotions. However, the neural underpinnings responsible for this unique, pair-specific experience remain largely unexplored. I will present work in which we used single nucleus RNA-sequencing to examine the transcriptional landscape of the nucleus accumbens (NAc) in socially monogamous prairie voles in peer or mating-based relationships. We identified cell type-specific gene modules that differ between relationship types and distinct gene modules that correlate with bond strength. Finally, we found that, regardless of relationship type, prairie vole pairs exhibit transcriptional synchrony with a partner. Transcriptional synchrony reflects an important role for the pair-specific social environment in shaping the NAc neurogenomic state and provides a potential biological mechanism by which shared social experience reinforces and strengthens relationships.

Session: Presidential Symposium 2

Honey bee learning and memory: Individuality is the key

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Honey bees have developed sophisticated learning abilities to face rapidly changing information about resources the colony needs for survival. To elucidate mechanisms that underlie different forms of learning, individuals can be evaluated in laboratory-based learning protocols. However, with the learning studied in the laboratory, the question becomes: how can these mechanisms be mapped to learning requirements in natural environments? We have studied how individuals differ in learning performance. Genotype is the major contributor to this variance. Queens mate with many drones and therefore produce multiple patrilines within any colony. Queens and (haploid) drones learn, and by pairing like-learning drone and queen phenotypes we have selected genetic lines for high and low performance on Latent Inhibition, whereby individuals are exposed to an odor without reinforcement such that subsequent learning about that now ‘familiar’ odor is reduced relative to a novel odor. We have shown how the brain changes encoding of familiar and novel odors, and we have used QTL mapping of crosses between high and low lines to identify genetic loci associated with high and low performance. One gene in particular has a major effect on expression of LI, and it appears to act as a gain control on the neural circuitry that implements Hebbian learning to reduce attention to familiar odors. We have now used selected lines to build colonies of pure and mixed genotypes to evaluate how they perform on a problem that required bees to exploit a known feeder or discover new ones that varied in position. High (LI) attention bees focused on the known feeder, whereas low attention bees discovered the new ones more frequently. We conclude that a mix of learning genotypes helps colonies to solve the exploration/exploitation foraging problem. Using selected genotypes as treatments in field colonies has.

A physiological perspective on the evolution of gestural communication

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Many animals communicate by performing elaborate gestures and other strange body movements. We see this most often in the context of sexual behavior, particularly when it comes to the peculiar displays that many taxa use to court mates and compete with rivals for opportunities to breed. One thing that makes this behavior so remarkable is that selection pushes it to extremes, making individuals perform in ways that we would otherwise think are unlikely (or even impossible). My research explores the mechanistic basis of these displays. I am especially interested in the way selection rewrites the systems that underlie performance to support innovations in gestural signaling. In this regard, my work spans multiple taxa, and I integrate approaches from fields of neuroscience, physiology, behavior, and evolution.

Session: Presidential Symposium 4

Circuit modules for locomotor speed control

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Locomotion is intimately linked to many behaviors, enabling social interactions, spatial navigation, and avoidance of danger. Therefore, the locomotor network must possess mechanisms that allow for precise tuning of the initiation of locomotion, its speed, and coordination to align with the desired behavioral outcome, such as slowly approaching a prey or rapidly escaping a predator. This precise tuning involves mechanisms ingrained within the connectivity of the locomotor circuits, combined with specific intrinsic neuronal properties and the integration of movement-generated proprioceptive feedback. Insights from our work, using adult zebrafish as a model system, have provided a new conceptual framework for understanding how locomotor movements are generated. Our research on zebrafish has provided a comprehensive framework for understanding the organization and function of motor circuits in adult vertebrates. I will summarize these insights with a particular focus on: (1) the modular design of the locomotor networks, which consist of three separate circuit modules that function as an intrinsic gear shift, controlling the speed of locomotor movements; (2) the role of motoneurons in the generation of locomotion, which are not passive recipients of motor commands but rather integral components of the neural circuits for motor behavior; (3) the recent discovery of an intraspinal proprioceptive organ that senses spinal cord movements; and (4) the descending commands controlling the start, duration, and speed of locomotion. Our results represent a rare example where the true organization of a vertebrate locomotion circuit has been uncovered at such a high level of resolution, bridging molecular, neuronal, synaptic, and circuit analysis with behavior in intact animals.

Cracking circuits with Connectomes: A Reverse Neuroethology perspective

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Recent advances in electron microscopy and computer-aided tracing capabilities have produced a new kind of Big Data: Connectomes. These maps of all the chemical synaptic connections between neurons in a brain are now publicly available for multiple species, including the entire worm nervous system, the fly brain and ventral nerve cord, and a patch of the mouse brain -- and maps in further species are forthcoming. Connectomes let us see the detailed wiring structure of the brain as never before, but what can these static maps actually tell us about brain function? I will discuss our lab's recent work analyzing the fly connectome and using it together with genetic, electrophysiological, and behavioral experiments to understand how sensory signals are converted to behavioral output across the nervous system. We find that connectomes are critical for understanding neural circuit implementation in the fly, but a wider knowledge of natural fly behaviors is still needed to fully interpret them.

An integrative approach to understand the logics of C. elegans motor control

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The ability of animals to generate goal-oriented motor behaviors ensures their survival. Applying connectomics, genetics, optogenetics, electrophysiology, and modeling, we have begun to reveal cellular mechanisms that drive the form, regulation, and adaptation of C. elegans motor behaviors. Our current progress implicates that the C. elegans motor circuit employs a few conserved organizational and functional logic of circuits that are functionally compression into a small number of neurons and seemingly shallow circuit layers.

Behavioral variables and neural mechanisms underlying helping behavior in mice

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Emotional contagion, the capacity to internally simulate another's emotional state, plays a crucial role in modulating prosocial behavior. This includes rescue behaviors, wherein individuals in a safe context intervene to release a trapped conspecific from an aversive context. However, little is known about how the perceived emotional arousal of the distressed individual influences the helper's behavior and the underlying neuronal mechanisms driving prosociability. This study explores the involvement of the dorsal hippocampus (dHPC), associated with contextual learning, and ventral hippocampus (vHPC), associated with emotional learning and memory, in modulating helping behavior in mice. Male and female mice were trained to rescue a distressed familiar conspecific of the same sex by opening a door under two conditions: the trapped mouse in a dry or flooded chamber. Cold water in the flooded chamber induced higher emotional arousal in the victim, resulting in reduced latency for rescuing by the helper. Silencing the dHPC, but not vHPC, prior to sessions significantly impaired rescue behavior, increasing latency and reducing the total number of helpers. C-fos expression after dHPC silencing suggested that emotional processing related areas were less engaged during the task. A decoder model, trained with 1-p calcium imaging and behavioral data, revealed that ~10% of dHPC cells exhibited correlated activity with helping behavior, showing increased activity after the release of the victim. These findings suggest that the dHPC is engaged during learning of rescue behavior, hinting at a contextual or episode-like memory component in the acquisition of this behavior. Utilizing single photon emission computerized tomography (SPECT) imaging, we further delineate the engagement of distinct brain regions across various stages of helping. Finally, through longitudinal monitoring of mice groups, we elucidate diverse social behaviors that underlie acts of helping.

On the nesting of dynamics in neuronal networks

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By strategy, functional neuroscientists tend to simplify the design of their experiments to better control factors or conditions that might complicate the interpretation of their observations. A great asset of neuroethological approaches is that they push us in the opposite direction, that is, they force us to consider the systems we study in the context and biological complexity in which they evolved to operate. In this talk, I will discuss a related issue, that concerns the dynamics of neuronal networks in behaving animals, and the wide range of time scales over which interesting things happen. Because these many time scales and dynamics concern on-going activity in the same circuits and neurons, one is sometimes forced to unfold or un-nest those dynamics from one another to hope to understand both their interplay and the roles they might each play. I will use examples from insect and fish olfaction, reptilian sleep and cephalopod camouflage to illustrate these points.

Oral Communications
Tuesday, 30 July 2024

Session: Plenary 2

Of fish and flies: studying the biological basis of sociality in two model organisms

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Sociality has been considered a major driver in brain and cognitive evolution. In my lab we combine the study of proximate causes (genes, hormones, neural circuits, cognitive processes) and ultimate effects (evolutionary consequences) of social behavior. For this purpose, we have been using two model organisms – zebrafish and fruit flies – to study the neural circuits and the genetic architecture of social behavior. In this talk, I will provide some examples of the work done in our lab in both model organisms. First, I will show how oxytocin plays a critical role in the development of sociality in zebrafish and how it interacts with the social environment during development to shape the emergence of adult social behavior. I will then, show how oxytocin is necessary and sufficient for complex social behavior in adult zebrafish, including social contagion of fear and emotion recognition. Finally, I will address the evolvability of sociality in zebrafish illustrated by an artificial selection experiment (currently in the F7) that experimentally produced a high-sociality zebrafish line. In the second part of my talk, I will present results on a study that investigates the genetic architecture of social cognition in Drosophila. We specifically address the question of social learning being a domain-specific or a general-domain cognitive process. We phenotyped social and asocial learning in the core lines of the DGRP panel, and we show that there is no phenotypic correlation between the two learning types and that GWAS revealed different genetic variants located in different genes associated with social and asocial learning. Finally, we show that most social learning-associated genes are expressed in the Drosophila mushroom bodies and functionally confirmed their involvement in learning using RNAi lines. Together these results highlight the potential of each model organism to address question related to the evolution of neural and genetic mechanisms underlying sociality.

Session: Invited Symposium 1

Category: Vision and photoreception

Polarization sensitivity in butterflies

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Butterflies can use reflected linearly polarized light from the wing surface of a conspecific to track down a mating partner or from the surface of leaves to find specific plants while foraging or ovipositing. During all these behaviours, polarized light is detected by the abundant polarization-sensitive photoreceptors in the butterfly compound eyes. At the same time, these photoreceptors mediate other visual qualities, such as colour or brightness contrasts, which results in a polarization signal for contrast enhancement and intraspecific communication that is not disentangled from colour or brightness information. We use single cell electrophysiology, anatomy and connectomics to identify the retinal substrate for polarization vision and the pathway for the propagation of information about polarization contrasts through the lamina in nymphalid butterflies. A subclass of ommatidia contains the photoreceptors with direct opponent synapses, with orthogonal polarization sensitivity maxima, that appear as ideal analyser pairs for polarization vision. In most cases, the pair is formed from a blue-sensitive long visual fiber with vertical sensitivity maximum, and a green-sensitive short visual fibre with horizontal sensitivity maximum. The signals from the green receptor converge with signals from all other photoreceptors in an ommatidium to single LMC interneurons in the lamina, which have no polarization sensitivity, as the signals from cells with phase-shifted polarization sensitivity maxima cancel out each other. The connectome data indicates that the only pathway from the polarization analyser pair to the brain is the blue long visual fibre, where the opponent input serves to enhance polarization contrast. The neural substrate for polarization vision in butterflies, which is not optimized for true polarization vision, independent of colour or brightness contrasts, provides a parsimonious explanation for many behavioural experiments involving linearly polarized light.

Session: Invited Symposium 1

Category: Spatial orientation and navigation

Importance of ocelli and DRA in Bumblebees foraging under different sky conditions

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Insects use polarized light (PL) patterns in the sky to navigate while foraging. However, the presence of clouds affects the degree of polarization (DoP) in the sky. In overcast conditions, the sun is not visible, and the DoP is eight times lower. It is currently unknown how this affects insect navigation. Studies suggest that the bee ocelli, in addition to the dorsal rim area (DRA, a specialized eye region sensitive to PL), can detect PL. We investigated how different sky conditions affect bumblebee foraging and the contribution of the ocelli and DRA. We recorded flights of bumblebees with all visual systems available (control bees), ocelli occluded, and DRA occluded inside a cage (3.3 m x 1.2 m x 1.2 m) with a sky view. During the experiment, we measured the DoP in the sky and grouped the sky conditions as clear sky (none or few clouds) or overcast (>80% occluded by clouds). We tested how the sky conditions and availability of visual systems influenced the path length and mean orientation of flights. Bees flew longer distances under overcast than clear skies ($F1.69=17$, $p0.05$ in all comparations), but in overcast, bees with DRA or ocelli occluded made longer flights than control bees ($p=0.04$, $p=0.007$, respectively). Bees with one of the structures occluded changed flight direction more often.

Session: Invited Symposium 1

Category: Spatial orientation and navigation

Dynamics of polarization-coding in the central complex of bumblebees

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Insects keep track of their heading through the activity of head-direction neurons in the central complex. The flight of bumblebees is characterized by alternating segments of translational motion and fast saccadic turns, resulting in a highly dynamic input to the compass system. Here we asked how such dynamic stimuli are processed. To answer this question, we recorded intracellularly from neurons of the central complex. We stimulated with a polarizer that could be rotated either continuously at rotational velocities between 30°/s and 1920°/s, or in a naturalistic way, according to the head orientation of a bumblebee during free flight (Boedekker et al. 2015).

We found that the coding of central complex neurons was highly dependent on rotation velocity and rotation direction. The faster the stimulus rotated, the later in the rotation cycle we observed the maximum response – indicating the intrinsic delay of the system. However, at lower rotation velocities, we observed the opposite effect – the neurons fired earlier than expected in the rotation cycle. To better understand these responses, we next presented polarized light with fixed angles of polarization and found that excitation of the neurons was often followed by inhibition upon termination of the stimulus, and vice versa, indicating an effect of spiking-history on the neuronal activity.

We next created population models that predicted responses either based on the angle of polarization and a time delay, or, additionally based on spiking-history. Quantitative comparison of the models and the measured responses confirmed the importance of spiking-history for the neuronal coding. Our population model also showed that the overall activity of the population peaked during fast changes of the stimulus orientation while it decreased, for stimuli with slow dynamics. These effects could facilitate a faster coding of the current heading during fast turns and might save energy during more straight segments of the flight.

Session: Invited Symposium 1

Category: Vision and photoreception

Polarization Vision in the Dark

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Until today, only one group of insects – the South African ball-rolling dung beetles, are known to steer by the dim pattern of polarization that forms around the moon. Their high sensitivity to the polarization of light is supported by a range of adaptations, from the surface of the eye to the retina and the brain. As a result, nocturnal beetles steer with the same precision and speed as their diurnal relatives.

Intracellular recordings reveal polarization-sensitive green photoreceptors in the most upper part of the dorsal eye of the nocturnal dung beetle Escarabaeus satyrus. Behavioural experiments verify the navigational relevance of this finding. To quantify the adaptive value of green sensitivity for celestial orientation at night, we obtained the polarization properties of the night sky in the natural habitat of the beetle. Calculations of relative photon catch reveal that under a moonlit sky, the beetle's green-sensitive photoreceptors can be expected to catch an order of magnitude more photons compared with its UV-sensitive photoreceptors. The green-sensitive photoreceptors – which also show a range of morphological adaptations for enhanced sensitivity – provide E. satyrus with a highly sensitive system for the extraction of directional information from the night sky.

In addition, compass neurons in the central complex of diurnal beetles are tuned only to the sun, whereas the same neurons in the nocturnal species switch exclusively to polarized light at lunar light intensities. Thus, these neurons alter their weighting according to ambient light conditions. This flexible encoding of celestial cue preferences relative to the prevailing visual scenery provides a simple, yet effective, mechanism for enabling visual orientation to the celestial pattern of polarized light at any intensity – in the field as well under the heavily light polluted skies over Johannesburg.

Session: Invited Symposium 2

Category: Methods and education

High Resolution Outdoor Videography of Insects Using Fast Lock-On Tracking

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Bees use vision and a tiny brain to find flowers and return home, but understanding how they perform these impressive tasks has been hampered by limitations in recording technology. Here we present Fast Lock-On (FLO) tracking. This method moves an image sensor to remain focused on a retroreflective marker affixed to an insect. Using paraxial infrared illumination, simple image processing can localize the sensor location of the insect in a few milliseconds. When coupled with a feedback system to steer a high magnification optical system to remain focused on the insect, a high spatial-temporal resolution trajectory can be gathered over a large region. As the basis for several robotic systems, we show FLO is a versatile idea which can be employed in combination with other components. We demonstrate that the optical path can be split and used for recording high-speed video. Furthermore, by flying a FLO system on a quadcopter drone, we track a flying honey bee and anticipate tracking insects in the wild over kilometer scales. Such systems have the capability of providing higher resolution information about insects behaving in natural environments and as such will be helpful in revealing the neuroethological mechanisms used by insects in natural settings.

Session: Invited Symposium 2

Category: Vision and photoreception

Neural circuits for active vision and natural behavior in the mouse

Author: Dr. Cristopher Niell, University of Oregon, Eugene, United States

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In the natural world, animals use vision to analyze complex scenes and enable a wide range of visually-driven behaviors, many of which require movement through the environment. However, much of our understanding of the neural circuit basis of vision is based on studies performed in stationary subjects performing artificial tasks in response to simple stimuli. In order to bridge this disconnect between how vision is actually used and how it is typically studied in the lab, we are investigating the neural circuits mediating ethological behaviors that mice perform. We have developed two behavioral paradigms, prey capture and gap crossing, that have provided insight into behavioral strategies and cell type-specific neural circuits for detection of relevant stimulus features within a complex and dynamic sensory environment.

An additional challenge in studying visual processing during natural behavior is the need to determine the visual input that an animal receives as it freely moves through its environment. We have therefore implemented novel experimental and computational methods, based on head-mounted cameras and chronically implanted silicon probes, that allow us to measure neural coding of the visual scene during free movement. These methods have revealed the impact of movement-related signals and active sampling on visual processing, and open up new directions for the study of vision in ethological contexts.

Session: Invited Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

Quantifying information during active movements in freely moving animals

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Why do animals move the way they do? I will consider the hypothesis that animals sometimes move in certain ways that maximize their ability to gather information about specific dynamic “states”, i.e. aspects of the environment, their movement, or parameters of their bodies. My group has developed computational tools that make it possible to determine which types of sensory information and movement motifs are required for acquiring specific types of information. I will present our mathematical and computational framework for applying these tools to trajectories of free moving animals as a means of understanding why animals make certain movement decisions, as well as generating hypotheses for what types of neural processing is likely to occur during free movement. Using this framework, we predicted that flying insects engaged in odor plume tracking ought to change course direction and groundspeed to estimate properties of the wind before they can deploy a wind-appropriate plume tracking strategy. In experiments that leverage remote optogenetic control of flying fruit flies’ olfactory experience, we see precisely the types of movements our theoretical framework predicted. Together, our theory and experiments provide theory and a compelling use case for how our approach can be used to probe active sensing behavior in free moving animals.

Session: Invited Symposium 2

Category: Spatial orientation and navigation

Uncovering spatial cognitive maps in zebrafish using brain-wide imaging in freely moving animals

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Cognition emerges when the brain transforms sensory information into abstract mental constructs or flexible internal representations. This internal cognitive representation of the external world is the basis for abstract thought, reasoning, and generalized intelligence. A prime example of this process is the mammalian ability to form spatial cognitive maps of the external environment. However, evidence for spatial maps (e.g. place cells) has yet to be convincingly identified in any species outside of mammals and birds. Thus, when and how spatial cognition emerged during evolution remains a central mystery of neuroscience. Using a state-of-the-art tracking microscope to image brain-wide activity at cellular resolution in freely swimming larval zebrafish, we computed the spatial information of neurons throughout the zebrafish brain. We find that the zebrafish telencephalon contains a network of place cells forming an internal representation of space. The place cell network in zebrafish exhibits striking similarities to mammals, as evidenced by multimodal integration of self-motion and visual input, experience-dependent refinement of the spatial map, and spontaneous offline reactivation of place cell ensembles. This recent discovery raises the possibility that spatial cognition arose in a compact circuit over 400 million years ago, setting up an initial condition for the subsequent elaboration and expansion of cognitive capabilities in mammals. Furthermore, the compact brain of larval zebrafish presents a unique opportunity to combine simultaneous neural recordings of the entire spatial computational network with the complete synapse-scale connectome of the underlying circuitry, to uncover the mechanistic principles underlying spatial cognition.

Session: Invited Symposium 3

Category: Other

Deciphering Neural Circuits for Vocal Communication: Insights from the Singing Mice

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My long-standing interest is to understand how circuits of interacting neurons give rise to natural, adaptive behaviors. Using vocal communication behavior across rodent species, my lab at CSHL pursues two complementary questions. How does the auditory system interact with the motor system to generate the fast sensorimotor loop required for vocal communication? What are the neural circuit modifications that allow behavioral novelty to emerge during evolution? In this talk, I will introduce you to the rich vocal life of the Costa-Rican singing mice. Next, I will describe a series of experiments that were performed to demonstrate the role of the motor cortex in controlling vocal flexibility in this species.

In closing, I will discuss our ongoing efforts to identify neural circuit differences between singing mice and lab mice using high-throughput connectomics. Together, by combining neural circuit analysis of a natural behavior with comparative evolutionary analyses across species, we stand to gain insight into the function and evolution of neural circuits for social behaviors.

This abstract is for an invited talk, which is part of the “Neural and behavioral principles structuring vocal interactions” symposium.

Session: Invited Symposium 3

Category: Other

Vocal communication in the naked mole-rat

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Highly organized social groups require well-structured and dynamic communication systems. Naked mole-rats form some of the most rigidly structured social groups in the Animal Kingdom, exhibiting eusociality, a type of highly cooperative social living characterized by a reproductive division of labor with a single breeding female, queen. Recent work from our group identified a critical role for vocal communication in the organization and maintenance of naked mole-rat social groups. Using machine learning techniques we demonstrated that one vocalization type, the soft chirp, encodes information about individual identity and colony membership. Colony specific vocal dialects can be learned early in life- pups that were cross-fostered acquired the dialect of their adoptive colonies. We also demonstrate that vocal dialects are influenced in part by the presence of the queen. Here, I discuss these findings and highlight our current work investigating how social and vocal complexity evolved in parallel in closely related species throughout the Bathyergidae family of African mole-rats.

Please note: This talk is part of the “Neural and behavioral principles structuring vocal interactions” symposium.

Session: Invited Symposium 3

Category: Auditory system and acoustic signaling

Tracking neural activity from auditory processing to precisely-timed vocal responses during zebra finch call interactions

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Vocal interactions involve auditory processing of conspecific acoustic signals and the selective initiation of context-appropriate vocal replies. This auditory-vocal transformation is the basis of the phylogenetically widespread phenomenon of vocal turn-taking, which can facilitate clear signal transmission and maintenance of social contact. However, the neural circuits that coordinate the timing and specificity of vocal responses to social sounds are not well understood. I will discuss this fundamental form of vocal control in zebra finches and present a behavioral assay for eliciting, disrupting, and quantifying call coordination. In addition, we use high-density electrode arrays to record neural activity across auditory and vocal premotor areas simultaneously, as birds listen to and produce calls. With this approach, we can trace sequences of neuronal firing across the pathway to determine how patterns evoked by specific acoustic signals drive vocal motor commands and coordinated call responses, within dynamic interactions.

Oral Presentation (as Co-Chair) for Invited Symposium: “Neural and behavioral principles structuring vocal interactions”

Session: Invited Symposium 3

Category: Auditory system and acoustic signaling

Deactivation of a frontal locus of vocal control in the bat modulates vocalization-related dynamics

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The frontal cortex of the highly social, echolocating bat Carollia perspicillata is believed to play an active role in vocal production and top-down modulation of auditory processing, as it receives projections from and projects to cortical and thalamic auditory regions. We investigate the causal role of this region in vocal production by reversibly deactivating a frontal region, the frontal auditory field (FAF), using the GABA A agonist muscimol. First, we found that local inhibition of the FAF dampens auditory-evoked local field potentials (LFPs) as well as spiking activity. Secondly, we found decreased gamma-band oscillatory power and an increase in the aperiodic (non-oscillatory or desynchronized) component of LFP activity in time windows directly preceding vocalization. Finally, we observed minor changes to spectral and temporal features of social calls, but not echolocation calls, emitted after frontal cortex deactivation. Together, these results suggest that the bat frontal cortex may be involved in structuring social vocalizations, while its inhibition leaves both the structure and frequency of the more innate echolocation calls unchanged.

Session: Invited Symposium 3

Category: Social behavior and neuromodulation

Stochastic dynamical systems model of vocal turn-taking and its development in marmoset monkeys

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The system of taking turns during vocal exchanges is fundamental to the communication of several animal species, yet their developmental origins and neural mechanisms remain elusive. Marmoset monkeys readily exchange vocalization when in acoustic contact with conspecifics. Moreover, their turn-taking capacity improves during development, decreasing the amount of overlap. We, therefore, used marmosets to explore the neural and developmental basis of turn-taking. To do that, we developed a stochastic dynamical systems model of marmoset monkeys based on the interactions among three neural structures ('drive,' 'motor,' and 'auditory') with feedback connectivity inspired by published physiological and anatomical data. The distinctive feature of our model is that it allows us to measure the amount of noise in the drive separately from the noise in the auditory system, where the noise plays a key role as a source of variability in the system. We fitted the stochastic model for each developmental period using vocal production recordings of infant marmosets. We found that during development, the noise level in the auditory system decreases, becoming noiseless after one month postnatal day. This noiseless period matches the timing of the significant improvement in marmoset capacity to avoid overlapping calls, suggesting that the increased signal-to-noise ratio in the auditory system is a major source for improvement in the turn-taking capacity.

This talk is part of "Neural and behavioral principles structuring vocal interactions" symposium.

Session: Invited Symposium 4

Category: Olfaction, taste and chemical sensing

The PROUST hypothesis: the evolution of the olfactory mind

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Complex behavior evolved upon a foundation of chemosensory processes: perception, action and memory. The importance of olfactory processes is particularly evident in spatial orientation. The exceptional size of olfactory receptor gene families allows phylogenetic analysis to common ancestors. Olfaction thus offers a unique tool to study the deep history of cognitive evolution. The PROUST hypothesis (perceiving and reconstructing odor utility in space and time) redefines the function of olfaction as a primary mechanism for orientation in the olfactory landscape. It offers testable hypotheses for previously paradoxical phenomena, such as the allometry of the main olfactory system, the secondary evolution of the vertebrate vomeronasal system, the later loss of hippocampal function in toothed whales and the importance of nasal respiration for memory consolidation in mammals. Olfactory cognition, as an ancient and universal trait in animals, may be the best paradigm to understand the nature of embodied cognition, enacted and embedded in an extended physical and social environment.

Session: Invited Symposium 4

Category: Learning, memory and cognition

Evolution of Memory: From Basic Foraging Decisions to Cognitive Map Construction

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Cognitive mapping builds internal representations of the world and is essential to episodic memory and mental imagery. Here we show how circuitry of basic foraging decision can be straightforwardly expanded for affective valuation and cognitive map construction in the agent-based foraging simulation, ASIMOV, reproducing likely potential evolution. Behavioral choice in foraging is governed by reward learning and motivation, which interact to assign subjective value to sensory stimuli. These qualities characterize foraging generalists that hunt in variable environments, and are precursors to more complex memory systems. ASIMOV's core decision network is based on neuronal circuitry of cost-benefit decision in the predatory sea-slug Pleurobranchaea californica. ASIMOV's forager affectively integrates sensation (olfaction, nociception), motivation (hunger), and learning to make cost-benefit decisions for approach or avoidance of prey. We further develop ASIMOV with the Feature Association Matrix (FAM), where simple episodic memory emerges from some of the most basic associative learning rules of classical conditioning. Spatial learning for obstacles and distant landmarks is enabled by a simple path integration system with homeostatic plasticity mechanisms. Addition of the FAM's spatial and episodic memory to ASIMOV's forager shows how the neuronal circuitry of foraging decision can serve as the framework for cognitive mapping in evolution.

Session: Invited Symposium 4

Category: Evolution and development

Genealogy vs. Convergence in Evolution of Integrative Systems: How to make a circuit, and a brain?

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How to make a neuron, a synapse, and a neural circuit? Is there only one 'design' for a neural architecture with a universally shared genomic blueprint across species? The brief answer is "No." I will provide evidence for neural systems' convergence and parallel evolution using several interdisciplinary approaches, from sequencing aboard oceanic vessels (Ship-Seq) and single-cell multiomics to behavior. Four early divergent lineages independently evolved distinct neuroid-type integrative systems from a nerveless common ancestor. One of these is a subset of neural nets in comb jellies (or ctenophores) with unique synapses; the second lineage is the well-known Cnidaria+Bilateria; the two others are non-synaptic neuroid primarily peptidergic systems in sponges and placozoans. Synapses also evolved more than once. Neuronal centralization and formation of the complex brain occurred at least 20 times independently. The first neural systems were peptidergic, with predominant volume transmission using at least several dozen signaling peptides. Multiple origins of neurons from secretory cells explain the observed molecular diversity of neural systems and non-synaptic integration of behaviors as the ancestral state. This scenario also explains the lack of homologs in neural systems across the earliest branching animal lineages. By integrating single-cell omics, we began to trace the genealogy of neurons toward reconstructing the Periodic System(s) of Neurons with predictive power by analogy to the Periodic System of Chemical Elements. We also revised the definition of neurons and their evolution from genetically heterogenous secretory cells at the top of behavioral hierarchies. Little-explored examples of convergent neuronal evolution in representatives of early branching metazoans provide conceptually novel microanatomical and physiological architectures of behavioral controls in animals with prospects of neuro-engineering and synthetic biology.

Session: Invited Symposium 4

Category: Evolution and development

Not much new in the last 500 – 600 million years?

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Functional networks of identified neurons in a molluscan model system are analogous to vertebrate cortex, basal ganglia and other pallial derivatives, reticular activating system, and spinal pattern generators for incentive, place, choice, posture, locomotion, arousal, and behavior. A common modular design characterizes nervous systems across the phyla. It answers the problems about sensory stimuli for choice, posture, locomotion, and arousal: “What is it, where is it, how do I like it, what should I do about it? And do it!” Decisions for action selection are transferred to modules that interpret them in terms of moment-to-moment posture and locomotion and primary motor output. The design is detectable in streamlined form in soft-bodied invertebrates with simpler networks that become greatly more complex in arthropods and vertebrates with segmentation, articulated skeletons, and more involved reproductive strategies. Examples are prominent in nervous systems of gastropods, insects, and vertebrates. The modular design may facilitate both natural evolution and computational modeling.

Session: Plenary 3

Learning to communicate by listening to others

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Juvenile zebra finches, a premier model of songbirds, learn to sing efficiently by vocal communications with adult tutors, but poorly from playback of tutor's songs (TS) during development. They first listen and memorize TS in the auditory learning phase, and then match their vocals to the memorized TS in the following sensorimotor learning phase. TS memories have been thought to be formed in the zebra finch higher auditory cortex, the caudomedial nidopallium (NCM). We found a subset of NCM neurons became to show highly selective auditory responses to TS playback by listening of tutor singing. The neurons in the locus coeruleus (LC), known to regulate attention and arousal state, project to NCM. Inhibiting LC projections in juvenile reduced NCM neuronal responsiveness to live tutor singing and impaired song learning, demonstrating LCNCM neuronal circuits integrates social information for song learning. We further found axonal projections in the song premotor area in the "song system", HVC from the NCM neurons responsive to playback of TS in juveniles. TS-responsive NCM neurons projected to two auditory areas, caudomedial mesopallium and HVCshelf, and Area X, a striatal part of the "song system" as well. Surprisingly axonal projection from TSresponsive NCM neurons was reduced specifically in HVC in the later stage of song sensorimotor learning phase. Ablating TS-responsive NCM neurons in juveniles, but not in adults, disrupts song learning. The density of axonal projections in HVC from TS-responsive NCM neurons negatively correlated with the level of song crystallization. Those suggest that dynamic axonal pruning may regulate timely vocal learning during development. Our studies have demonstrated neuronal circuits from/to the zebra finch higher auditory cortex, NCM, regulate efficient song memorization by vocal communications with adult tutors and auditory-memory guided song learning in well-orchestrated developmental auditory and sensorimotor learning period.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

The transcriptional logic of ant odorant receptors

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Insects and mammals have independently evolved odorant receptor genes that are arranged in large genomic tandem arrays. In mammals, each olfactory sensory neuron chooses to express a single receptor in a stochastic process that includes substantial chromatin rearrangements. Here we show that ants, which have the largest odorant receptor repertoires among insects, employ a different mechanism to regulate gene expression from tandem arrays. Using single nucleus RNA sequencing, we found that ant olfactory sensory neurons choose different transcription start sites along an array, but then produce mRNA from many downstream genes. This can result in transcripts from dozens of receptors being present in a single nucleus. Such rampant receptor co-expression at first seems difficult to reconcile with the narrow tuning of the ant olfactory system. However, RNA fluorescence in situ hybridization showed that only mRNA from the most upstream transcribed odorant receptor seems to reach the cytoplasm where it can be translated into protein, while mRNA from downstream receptors gets sequestered in the nucleus. This implies that, despite the extensive co-expression of odorant receptor genes, each olfactory sensory neuron ultimately only produces one or very few functional receptors. Evolution has thus found different molecular solutions in insects and mammals to the convergent challenge of selecting small subsets of receptors from large odorant receptor repertoires.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

Sensory neurobiology of egg laying preference in Aedes aegypti mosquitoes

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Oviposition (egg laying) is a critically important behaviour for many animals, including disease-vectoring mosquitoes. In this presentation, I will outline our lab's work to understand the egg laying preferences of the yellow fever mosquito, Aedes aegypti. Following a blood-meal, a female mosquito must decide where to deposit eggs so as to maximize the survival of her offspring. Larval and pupal mosquitoes are fully aquatic and so egg-laying decisions by the adult female binds offspring to a specific body of water for all stages of early development. The female mosquito must first identify water from a distance and then evaluate the physical and chemical properties of a potential egg laying sites using contact sensory systems, ensuring that it is optimal for the survival of her aquatic offspring.

We have explored two sensory modalities that control egg laying in Ae. aegypti: mechanosensation (to evaluate surface texture, ensuring that eggs do not prematurely fall into the water) and chemosensation (to evaluate the chemical composition of the water, leading to maximal survival probability for their offspring). Using quantitative egg-laying assays, we show that mosquitoes have a dose-dependent positive preference for rough textures, and a dose-dependent aversion to salt. When presented with conflicting cues, the animals integrate these two orthogonal cues as they seek to maximize fitness in the next generation.

We are now investigating mosquito egg-laying using quantitative real-time behaviour assays coupled with molecular genetics, CRISPR-Cas9 generated mutations, and cell-type specific driver lines that can visualize and manipulate neural activity. We developed a strategy to label molecularly-defined populations of sensory neurons for sorting and sequencing in order to identify candidate receptors for the mechanical and chemical cues that guide egg-laying, ultimately seeking to understand mosquito behaviours at the behavioural, circuit, and molecular level.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

Adenosine signaling in glia modulates metabolic state-dependent behavior in Drosophila

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An animal's metabolic state strongly influences its behavior. Hungry animals must engage in food seeking and feeding behaviors in order to restore their energy balance. Activity of the neuronal networks underlying these behaviors themselves consume energy. Indeed, neuronal excitability and synaptic transmission are among the most energy expensive processes in an animal's body. Yet neurons do not uptake glucose -their main energy source- from the circulating blood and can neither store it. This highly conserved function is rather carried out by glial cells. In addition to their role as energy suppliers of the nervous system, glia can sense and modulate neurotransmission and animal behavior based on more recent work in various invertebrate and vertebrate models. In this present study, we show that the metabolic state of hungry Drosophila melanogaster can be transmitted to glial cells by adenosine. Adenosine signaling in glia modulates their intracellular calcium activity which leads to modification of neural activity in response to food odor and ultimately of food foraging and feeding behaviors. Interestingly, adenosine signaling in specific glial subpopulations has different, in some cases opposite, effects on their activity and on fly behavior. Taken together, we provide a new mechanism that glial cells can use to sense the metabolic state of the animal and modulate its behavior accordingly.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

Cave odours as possible destination cues in the long-distance navigation of the Australian Bogong moth Agrotis infusa

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The Bogong moth Agrotis infusa is a long-distance nocturnal migrant whose navigational ability still remains only partly understood. They travel up to a thousand kilometres to the Australian Alps where they seek out the shelter of isolated cool alpine caves, thereby escaping the approaching summer heat of their breeding grounds. Once in the caves, they enter a state of dormancy over the coming summer (known as aestivation). How they find their aestivation caves is still a mystery. Our hypothesis is that at the end of their journey, during the final “pin-pointing phase” of their long-distance navigation, they use their olfactory system to home in on their cave destination. We investigated the olfactory system of the Bogong moth to address this hypothesis by using odour collections from the caves (analysed in a gas chromatograph-mass spectrometer), electrophysiological recordings from the antennae and behavioural experiments. When analysing cave odours, one compound - (Z,E)-9,12-tetradecadienyl acetate - was found that males strongly react to electrophysiologically and both sexes behaviourally. During the spring migration towards the caves they are significantly attracted to this compound, but when leaving the caves on their return journey in the autumn, they are indifferent to it. The origin of this compound remains unknown. However, the three likeliest sources are the moths themselves, microbial activity in the soil or one or both species of parasitic mermithid nematodes that live only in these caves.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

Unraveling the effects of the short neuropeptide F (sNPF) in Winter bees

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Neuropeptides constitute an important class of signaling molecules in the nervous system. The neuropeptide F (NPF) and the short neuropeptide F (sNPF) of invertebrates have similar functions as the neuropeptide Y (NPY) of vertebrates and modulate several biological processes such as circadian rhythms, learning, memory, and feeding. In the honeybee, a social insect in which workers search for food in response to colonial needs, sNPF represents a key molecule driving foraging-related behaviors during periods of food-search activity (springtime & summer). These behaviors include food intake, sucrose sensitivity, olfactory perception, and visual memory formation. Yet, it is unclear whether sNPF exerts the same effects in winter bees, which do not engage in foraging activities and remain in their hive performing thermoregulatory duties due to unfavorable weather conditions. Here we studied the effects of sNPF in winter bees, via topical exposure to the neuropeptide. We asked if increasing sNPF levels would rescue summer-like states and hence enhance different appetitive behaviors in environmental conditions inconsistent with this enhancement. Increasing levels of sNPF in winter bees increased sucrose responsiveness yet failed to achieve a similar effect for appetitive olfactory spontaneous responsiveness, olfactory learning, memory, and food consumption. Our results demonstrate that sNPF may seasonally affect honeybees. During summer, the sNPF system modulates outdoor behaviors that will ensure colony preparation and survival for the winter (e.g. searching for food). During winter, the system may regulate only traits that guarantee individual survival within the hives (e.g. gustatory responsiveness). Increased gustatory sensitivity may ensure that bees will perceive and hence feed on any available resource within their hives ensuring their survivorship. Our results suggest that the sNPF signaling system is dynamic and is impacted by external factors such as seasonality.

Session: Participant Symposium 1

Category: Olfaction, taste and chemical sensing

Drosophila melanogaster eavesdrops on a yeast quorum-sensing signal to locate food sources

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Drosophila melanogaster and Saccharomyces cerevisiae feed on decaying fruits, a food source rich in sugar. It has been hypothesized that D. melanogaster uses volatiles produced by fermenting yeasts to locate food sources, whereas yeasts might rely on the insects to disperse to new food patches. To test whether D. melanogaster is attracted to volatiles produced by S. cerevisiae, we measured the innate attraction of D. melanogaster for various fruit juices and fermenting products. Our results revealed red wine as the most attractive product tested. By identifying the predominant volatiles in wine, we observed D. melanogaster's pronounced attraction to 2-phenylethanol (2-PE), a quorum-sensing molecule produced by S. cerevisiae in times of high density and nutrient depletion. To assess the role of 2-PE in mediating D. melanogaster's attraction to S. cerevisiae, we generated mutant aro8/aro9Δ yeast that lacks the ability to produce 2-PE. Over days, D. melanogaster's preference for wildtype over mutant yeast increased with rising 2-PE levels. Adding equivalent levels of 2-PE to aro8/aro9Δ yeast eliminated this preference. Additionally, 2-PE-supplemented mutant yeast was preferred over untreated yeast, showing 2-PE potency in influencing yeast preference. To elucidate the olfactory pathway through which D. melanogaster detects 2-PE, we performed calcium imaging experiments, revealing that D. melanogaster detects 2-PE through the Or67b pathway. However, 2-PE preference varies in D. melanogaster strains and Drosophila species, indicating that the trait developed recently in evolutionary history. Altogether, these data suggest that D. melanogaster has evolved the ability to locate profitable food sources by eavesdropping on the chemical signals used by S. cerevisiae to communicate their quorum status to their conspecifics. S. cerevisiae might produce 2-PE not only to communicate with their conspecifics but also to recruit D. melanogaster as a dispersal vector to new habitats.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

Wind Gates Search States in Free Flight

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For organisms tracking the plume of a volatile chemical cue to its source, information from the flow surrounding them provides crucial information for success. Swimming and flying searchers begin tracking plumes first with a surge into the oncoming wind or tide, and cast cross-flow after passing through the chemical plume. This "cast and surge" dynamic is nearly ubiquitous across taxa searching for the source of a smell in both the aquatic and terrestrial ecosystems. In nature however, the wind or water currents may not always provide a clear directional cue for an organism. Either when they are turbulent or very still. How then should organisms deploy a viable search strategy without a reliable rheotactic cue? Here we answer this question by developing an optogenetic paradigm for free flying Drosophila that allows us to deliver millisecond precise, identical olfactory experiences independently of manipulations to the wind environment. We show that in addition to the well-described "Cast and Surge" behavior in laminar wind, flies have another complementary plume tracking strategy specialized for still air. This "sink and circle" style of search is dominated by two features- rhythmic unidirectional saccades and lowering their altitude. "Sink and circle" is a proximal search strategy and is an evolutionarily convergent strategy with canids and murids which alternate between bouts of raising their snouts to sample the air above and down close to the viscous boundary layer when tracking odor plumes. We also investigate an overlooked component of odor navigation- how do flies measure properties of wind while flying? We show that flies use an active sensing maneuver in the milliseconds just prior to surging or circling which is consistent with recent control theoretic analysis of how flying organisms can uniquely gauge properties of the wind without magnitude calibrated sensory systems.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

Beyond the buzz: How mosquitoes combine visual and acoustic cues to navigate within swarms

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Navigating within a large collective, coordinating with other group members while avoiding collisions, is a challenge faced by species across animal kingdom, from stampeding wildebeests to nest-building ants. Flying insects that form mating swarms are no exception; they must successfully maneuver complex aerial environments to find a mating partner. However, the sensory mechanisms that enable swarming individuals to navigate these environments remain largely unknown. We investigated how Anopheles coluzzii, key vectors of the pathogens causing malaria in Africa, find their mates while avoiding collisions with other swarming individuals. While it is well-established that male mosquitoes use the acoustic signals of female flight tones for mate localization, our research uncovers the role of visual cues in swarm navigation. Employing a virtual reality flight simulator with tethered mosquitoes, we found that acoustic cues enhance the ability of male mosquitoes to track visual objects simulating nearby conspecifics. Additionally, we found that mosquitoes can independently adjust their flight responses to visual objects and that this behavior is similar to the collision avoidance response in free-flying mosquitoes within swarms. Our findings provide compelling evidence that mosquitoes can integrate short-range visual and acoustic cues for efficient mate tracking while avoiding collisions, offering new insights into the complex sensory world of swarming mosquitoes.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

Vision vs. Echolocation: Navigational Strategies of Egyptian Fruit Bats Amidst Sensory Conflict

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Egyptian fruit bats, Rousettus aegyptiacus, present a distinct opportunity to unravel the relative contributions of two distal sensing systems, echolocation and vision, in guiding spatial navigation. This species relies heavily on rod vision but has also evolved lingual echolocation to localize objects in the dark. Auditory and visual localization are anchored to the head, but the bat's active control over sonar beam aim, ear, and eye position can influence sampling of sensory space. We investigated the relative weighting of echolocation and vision in free-flying Egyptian fruit bats, by manipulating auditory and visual inputs as they navigated. In this experiment, we used stereo IR video and microphone array recordings to quantify the bat's trajectory, head direction and sonar beam aim as it flew to a landing perch. We introduced sensory conflict by equipping bats with goggles fit with prisms that shifted visual images by 23 degrees to the left or right. In control trials, prisms were replaced with clear or light-blocking lenses. Initially, bats flew in the direction of the prism shift, but gradually, they adjusted their flight paths to accommodate for altered sensory input. To explore the role echolocation played in this adaption, we 1) alternated placement of left and right prisms and 2) disrupted the bats' use of echolocation with sound attenuating ear molds. In both dark and alternating prism trials, bats were able to land, but with some difficulty. During these trials, the bats echolocation was often directed at the true perch location, even when their head orientation and flight path were influenced by the misperceived position of the perch. When both vision and echolocation were disrupted (prisms and ear molds) bats were no longer able to land. These findings indicate that Egyptian fruit bats predominantly rely on visual cues for navigation; however, echolocation provides a reliable backup system when visual information is distorted or absent.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

How night-flying mosquitoes rapidly evade invisible looming objects

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To detect and escape from a looming threat, night-flying insects must rely on other senses than vision alone. Nocturnal mosquitoes have been described to escape looming objects in the dark, but how they achieve this is still unknown. Here, we show how night-active female malaria mosquitoes escape from a rapidly looming object that simulates the defensive action of a blood-host. By combining videography-based automatic tracking with numerical simulations of the attacker-induced airflow, we first show that night-flying mosquitoes use airflow-sensing to detect the danger and trigger their escape. Secondly, by combining these data with mechanistic movement modelling, we unravelled how mosquitoes control their escape manoeuvres: they actively steer away from the danger, and passively travel with the bow-wave produced by the attacker. Our results demonstrate that night-flying mosquitoes escaping from a looming object use the object-induced airflow both to detect the danger, and as fluid medium to move with for avoiding collision. This shows that the escape strategy of flying insects is more complex than previous visually-induces escape flight studies suggest. As mosquitoes are average-sized insects, a combined airflow-induced and visual-induced escape strategy is expected to be common amongst millions of flying insect species. Also, our research helps explain the high escape performance of mosquitoes from counterflow-based odour-baited mosquito traps. It can therefore provide new insights for the development of novel trapping techniques for integrative vector management.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

Touch inhibits feeding through a neural bottleneck in C. elegans: a window to a biological information compression system

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A neural bottleneck is a network motif widespread across species where multiple neurons project onto a smaller subset. This motif suggests that the network compresses information encoded in the incoming signals. However, measuring such information compression remains challenging, as most systems do not allow observation of all neurons participating in the bottleneck. The pair of RIP neurons in the nematode C. elegans represents a simple implementation of a neural bottleneck where such measurements are possible. RIP neurons receive converging sensory inputs and provide the only connections to the pharyngeal network controlling feeding through gap-junctions with the pair of pharyngeal I1 neurons. We developed a high-throughput assay to supply substrate vibrations as touch stimulus while automatically reading out the feeding behavior in populations of freely moving C. elegans. We found that vibrations inhibit feeding and trigger an escape response in an intensity dependent manner. These responses are abolished in the touch defective mutants mec4(u253) confirming that the Touch Receptor Neurons (TRNs) constitute the input layer in our paradigm, as mec4 encodes a mechanotransducer channel subunit exclusive to TRNs. We then tested the requirement of the RIP-I1 bottleneck by genetically ablating I1 neurons using the human caspase interleukin-1β-converting enzyme (ICE). Feeding suppression, but not the escape response, is abolished in these animals suggesting that the touch signal flows through the bottleneck. Finally, we will show our progress to measure how the intensity of the touch stimulus is encoded and transformed at the different layers of the network using calcium imaging in single neurons in freely moving animals expressing GCaMP8f in the TRNs, RIP and I1 neurons respectively. This behavioral paradigm provides a unique window for exploring the role of neural bottleneck networks, potentially shedding light on generalizable principles of information compression.

Session: Participant Symposium 2

Category: Motor systems, sensorimotor integration, and behavior

The brains of snapping shrimp are protected from shock waves by their helmet-like orbital hoods

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Shock waves are supersonic high-amplitude pressure waves that cause barotrauma by transferring kinetic energy to the tissues of animals. Using their snapping claws, snapping shrimp create cavitation bubbles that release shock waves when they collapse. Snapping shrimp produce and are exposed to shock waves continually, so we asked how snapping shrimp survive frequent, close encounters with shock waves powerful enough to kill other animals. We tested if the brains of snapping shrimp are protected from shock waves with a helmet-like extension of their exoskeleton, the orbital hood. Using behavioral trials, we found shock wave exposure slowed shelter-seeking and caused a loss of motor control in Alpheus heterochaelis from which we had removed orbital hoods but did not significantly affect shrimp with unaltered orbital hoods. Following shock wave exposure, A. heterochaelis without orbital hoods showed increased apoptosis in their brains immediately after exposure and for at least 72 hours thereafter, whereas animals with unaltered orbital hoods did not. Shock waves thus have the potential to harm snapping shrimp but may not do so under natural conditions because of protection provided to shrimp by their orbital hoods. Next, using pressure recordings, we discovered the orbital hoods of A. heterochaelis protect against barotrauma by dampening shock waves. Sealing the anterior openings of orbital hoods diminished how much they altered the magnitudes of shock waves, which suggests these structures dampen shock waves by trapping and expelling water so that kinetic energy is redirected and released away from the heads of shrimp. Using µCT and TEM, we discovered that orbital hoods also have structural modifications associated with their ability to dampen shock waves. Our results indicate orbital hoods mitigate blast-induced neurotrauma in snapping shrimp by dampening shock waves, making them the first biological armor system known to have such a function.

Session: Participant Symposium 3

Category: Evolution and development

Dynamics of adaptability and constraints in the evolution of a learning and memory circuit in Heliconiini butterflies

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Central neural circuits integrate sensory and internal information to cause a behavioural output. Evolution modifies such circuits to generate adaptive change in sensory detection and behaviour, but it remains unclear how selection does so in the context of existing constraints. Here, we explore this question by analysing evolutionary dynamics in the insect mushroom body circuit as well as connected circuits, particularly the central complex. While the central complex seems to be largely conserved in term of size and shape, there is huge diversity in the mushroom body circuit. However, an empirical framework of how evolution modifies the function and architecture of these circuits is largely lacking. To address this, we leverage the recent radiation of a Neotropical tribe of butterflies, the Heliconiini (Nymphalidae), which show a massive amount of variation in mushroom body size over comparatively short phylogenetic timescales, linked to specific changes in foraging ecology, life history and cognition. We combined immunostainings of structural markers and neurotransmitters as well as neural injections with comparative, quantitative datasets to understand the mechanism by which such an extensive increase in mushroom body size is accommodated through changes in internal and external circuitry. Inside the mushroom bodies, we identified that only some Kenyon cell populations expanded with a higher rate than others, in those Heliconiini faced with specific cognitive demands for their foraging ecology. We also identified that feedback neurons external to the mushroom body show a large increase in cell number. This is accommodated with large conservation inside central complex and peripheral circuitries, with highly intriguing specific differences. Our results demonstrate an interplay of evolutionary malleability inside the mushroom body lobes and functional constraints inside connected circuits as an evolutionary pathway guiding adaptation in cognitive ability.

Session: Participant Symposium 3

Category: Evolution and development

Selection for sociality drives divergent brain transcriptomes in zebrafish

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It is possible to observe similar behavioral phenotypes like pair bonding or maternal care, across diverse animal species. This leads to the question of whether shared behaviors have a common genetic basis – a behavioral toolkit, that is conserved and reused throughout evolution to embed similar behaviors. Studies suggest that behavioral phenotypes are associated with changes in expression of multiple genes across different brain regions, creating regulatory networks at molecular and functional levels. In this work we computationally explore the genes and gene regulatory networks underlying the evolution of sociality. An artificial selection experiment for high and low sociality lines in zebrafish has been ongoing in the lab, using a social preference paradigm (i.e., video of a mixed-sex conspecific shoal vs video of moving circles), and the individual preference to associate with a shoal or the circles was quantified. After 3 generations (F3), the lines selected for sociality started to diverge significantly in social preference from the other lines (circle and control). The transcriptomic profiles of these three lines were analyzed in different brain regions, and the results suggest the presence of differentially expressed genes associated to each selected line. Further, a Weighted Correlation Networks Analysis (WGCNA) has been applied, which allowed us to find clusters of genes specific to each behavioral line as well. Throughout the next generations, up to F7, the selective pressure increased. Considering this, we looked for conserved patterns in gene expression and regulation underlying sociality at single-cell resolution.

Keywords: Artificial selection, Zebrafish, Social Behavior, Transcriptomics, WGCNA, single-cell

Session: Participant Symposium 3

Category: Evolution and development

Evolutionary Repurposing of the Functional Role of the Optic Tectum in Cavefish

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Among the many adaptations that Astyanax mexicanus cavefish populations have evolved to cope with the drastic changes in the environment are significant changes in their sensory systems. Eye loss and expansion of other sensory systems have emerged in the many different Astyanax cavefish populations. Despite years of investigation of the evolution of the sensory systems, the evolution of neural circuit dynamics and brain computations remains unknown.

For this, we used transgenic fish expressing GcaMP6s (from Keene lab) from Pachón and Molino caves, and Surface-fish populations to image activity in the optic tectum. Despite the eye loss in cavefish, the optic tectum, their primary visual area, only shows a reduction in size of ~20%. Its spontaneous activity showed no difference from that of surface fish. These raised the possibility that the tectum was repurposed for functions other than vision. Therefore, we set to explore the sensory-induced and spontaneous activity of the tectum in both surface fish and cavefish populations to get insights into the neural basis of sensory adaptation and evolution.

Our preliminary results showed similar spatiotemporal spontaneous dynamics of neuronal activity in both cavefish and surface fish. In zebrafish, the spontaneous dynamics reflect the functional connectivity of the tectal circuit, adapted to improve visual detection of vital stimuli (e.g., prey). We found that the former visual area in the cavefish tectum responded to small water-jet stimuli restricted to an area around the head. The response to the water-jets retained a similar spatial representation to that of the surface fish retinotopic map. Therefore, we hypothesize that the optic tectum was evolutionarily repurposed to encode water flow around the larva's head, retaining the functional connectivity of the ancestor retinotopic map of surface fish. The visual-like representation of the water flow information allows cavefish to navigate, orient, and prey in the caves.

Session: Participant Symposium 3

Category: Evolution and development

The neural basis of defensive behaviour evolution in Peromyscus mice

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Evading imminent predator threat is critical for survival. Effective defensive strategies can vary, even between closely related species. However, the neural basis of such species-specific behaviours is still poorly understood. Here we find that two sister species of deer mice (genus Peromyscus) show different responses to the same looming stimulus: P. maniculatus, which occupy densely vegetated habitats, predominantly dart to escape, while the open field specialist, P. polionotus, pause their movement. This difference arises from species-specific escape thresholds, is largely context-independent, and can be triggered by both visual and auditory threat stimuli. Using immunohistochemistry and electrophysiological recordings, we find that although visual threat activates the superior colliculus in both species, the role of the dorsal periaqueductal gray (dPAG) in driving behaviour differs. While dPAG activity scales with running speed and involves both excitatory and inhibitory neurons in P. maniculatus, the dPAG is largely silent in P. polionotus, even when darting is triggered. Moreover, optogenetic activation of excitatory dPAG neurons reliably elicits darting behaviour in P. maniculatus but not P. polionotus. Together, we trace the evolution of species-specific escape thresholds to a central circuit node, downstream of peripheral sensory neurons, localizing an ecologically relevant behavioural difference to a specific region of the complex mammalian brain.

Session: Participant Symposium 3

Category: Metabolism, biological rhythms and homeostasis

The blood-brain barrier in stasis: neurovascular changes during mammalian hibernation

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Mammalian hibernation presents many unique physiological challenges that have been met with flexible adaptations to nearly every organ system. The thirteen-lined ground squirrel (Ictidomys tridecemlineatus) hibernates for 5-7 months in the winter. During hibernation, squirrels cycle between bouts of torpor lasting 3-14 days, when they lower their heart rate, core body temperature, metabolism, and breathing rate to 1-5% of active levels, and interbout arousal (IBA), when squirrels temporarily (12-48 hours) awaken and return to an active-like state. This relatively rapid and extreme modulation of cardiovascular output provides an opportunity to study neurovascular function at the extreme ends of perfusion. Here, we use tracer injection experiments and in vivo GRIN lens imaging to study BBB function and cerebral hemodynamics. Tracer injections show that that BBB tight junctions surprisingly tighten during hibernation states in brain cortex, but hypothalamic tight junctions maintain basal permeability during hibernation. Transcytosis is suppressed in all brain regions during torpor. Live blood flow imaging across physiological states shows no discernible vasoconstriction in brain capillaries, despite drastic fluctuation in blood flow velocity between torpor and IBA. This suggests uncoupling of endothelial cell tight junction regulation from blood flow during torpor, which may be dynamically regulated during the transition to IBA. However, virally transduced GCaMP6s expression in excitatory neurons reveals that electrically inactive torpid cortical neurons only resume firing when body temperature reaches ~20C. This is far warmer than has been demonstrated for resumption of neuronal firing in other brain regions, and cortical neurons only resume firing once local blood flow velocity has plateaued. This could represent a convergent point in the rewarming process where sufficient perfusion has occurred, and nonessential brain areas may resume activity.

Session: Participant Symposium 3

Category: Metabolism, biological rhythms and homeostasis

Artificial light at night alters cricket stridulation patterns even in semi-natural environments

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Most organisms, including insects, synchronize their daily activity patterns and internal processes to the natural cycle of day and night. Such synchronization is used for internal timekeeping and for adapting to changing environmental conditions. The circadian rhythm is thus sensitive to alterations of light and darkness. Artificial light at night (ALAN), a widespread anthropogenic pollutant, masks the natural day–night cycle and was reported to negatively impact animals at various levels. However, our understanding of the deleterious impacts of ALAN on nocturnal insects is mostly based on laboratory studies and remains limited. We studied the effect of different ALAN intensities on stridulation behaviour in crickets exposed to semi-natural conditions. Adult, male field crickets, Gryllus bimaculatus, were housed in outdoor terraria and subjected to shaded natural lighting and natural temperature cycles. The crickets were exposed to one of several light treatments: daylight:darkness (LD); daylight:ALAN of 2, 5, 15, 100, or 400 lux; and constant light of 1500 lux. The crickets' stridulation behaviour was continuously recorded for 14 consecutive days and nights, and their daily activity periods were compared among groups. While crickets under LD displayed nocturnal stridulation with a rhythm of 24h, exposure to ALAN conditions resulted in a light-intensity-dependent increase in the proportion of crickets demonstrating free-run behaviour. Moreover, the variance and medians of the demonstrated activity period differed significantly between the LD and ALAN treatments >100 lux (Kruskal-Wallis test, $p<0.05$). The ALAN-induced changes in the crickets' timing of activity resulted in a decrease in the overall population synchronization. Our results revealed an ALAN-induced loss of timekeeping of the individual insects, leading to an ALAN-intensity-dependent desynchronization of the population, even under semi-natural conditions, thus confirming ALAN as an ecological threat.

Session: Participant Symposium 4

Category: Vision and photoreception

Functional organization of visual responses to luminance and polarization stimuli in the octopus

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Cephalopods have a complex visual system, with camera-type eyes and a large brain. Furthermore, they can see polarization, or the angle of oscillation of a light wave, which humans cannot detect. Most of our understanding of polarization sensitivity in the cephalopod visual system comes from studies investigating retinal responses and behavioral discrimination tasks, leaving many unknowns in how polarization information is processed in the central brain. To investigate how the octopus brain encodes different aspects of the visual scene we are using two-photon calcium imaging to record large-scale organization of neural activity in the optic lobe of Octopus bimaculoides. By presenting luminance-based stimuli we discovered localized receptive fields, retinotopic organization, and ON and OFF processing pathways. To further understand how octopuses process polarized light we developed and calibrated a display to present stimuli that vary in polarization angle rather than luminance. We find spatially localized receptive fields in response to changes in polarization angle, and these receptive fields have a retinotopic organization similar to that generally observed for standard luminance stimuli. In addition, we find distinct yet overlapping spatial patterns of activation for polarized light compared to luminance. Lastly, our data suggests that vertically polarized and horizontally polarized light may be processed in different areas of the optic lobe. The results show a highly organized representation of polarization stimuli, beyond that observed in other species, which could support the role of polarization in image-forming vision in cephalopods. These findings will also enable future studies investigating the circuit mechanisms that enable polarization vision in cephalopods.

Session: Participant Symposium 4

Category: Vision and photoreception

The effect of the whole-genome duplication on vision: How does the common barbel (Barbus barbus) see?

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Teleosts outstand among all vertebrates in the number of visual pigments and photoreceptor types. This extraordinary development was also facilitated by the teleost-specific whole genome duplication that occurred approximately 350 Mya. Here we explore teleost species that recently underwent subsequent whole genome duplication – the common barbel (Barbus barbus). This event was accompanied by hybridization (i.e., it was allopolyploidization) and resulted in 14 opsin genes found in the genome, an unusually high number even among teleosts. Does the expansion of opsin gene repertoire result in better visual abilities? We show opsin expression profiles of adult specimens and larvae at different developmental stages. Within three opsin classes (UV-sensitive SWS1, blue-sensitive SWS2, and green-sensitive RH2), we found opsin pairs with a clear temporal division of function – larval and adult genes. Furthermore, the switch in expression from larval to adult gene copy in these opsin classes was synchronized – such effect has never been reported for a polyploid species. We investigated if such a division of function can be adaptive. We predict which opsin proteins differ in spectral sensitivity based on the amino acid substitutions in the key tuning sites. Are absorption spectra of opsins tuned to match the light available in the environment? We localise and visualise the opsin gene expression in adult retinae via fluorescence in situ hybridization and discuss its functional consequences – namely if the presence of more opsins in the retina enhances colour vision or sensitivity of the visual apparatus. Finally, we present the latest results of single nuclei RNA sequencing performed on the adult retina to reveal how individual cells utilise vision-related genes acquired by the polyploidization event.

Session: Participant Symposium 4

Category: Vision and photoreception

The frog's approach to colour vision in the dark: retinal computations and connections

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Amphibians are unique among vertebrates in possessing a rod photoreceptor sensitive to blue in addition to the most widespread one sensitive to green, and they can use this rod pair for chromatic discrimination near the absolute visual sensitivity threshold in specific behavioural contexts. It is unclear how the 'blue' rods arose in the amphibian lineage, and understanding how they are incorporated into the retinal processing pathway would provide meaningful insights into the potential and constraints for evolutionary rewiring of retinae and for expanding the repertoire of visual abilities available to an animal. So, how is this "colour vision in the dark" encoded in the retina, and how is the retinal circuit wired to enable it?

We are tackling these questions at the physiological and anatomical levels. On one hand, we are using high-density multielectrode arrays to record responses of retinal ganglion cells (RGCs) in adult Xenopus to low intensity stimuli. Our initial experiments showed that a few RGCs appear to encode wavelength by subtly suppressing the ON component of otherwise typical, green-dominant ON-OFF responses. This modulatory effect of the 'blue' circuit on the 'green' circuit entails a potential explanation for how colour biased behavioural responses in dim light might be achieved. Following up on these insights, we are expanding the battery of stimuli to better understand how this putative ON-suppressive circuit operates. On the other hand, we are exploring its anatomical architecture in a serially sectioned electron microscopy dataset. By tracing the synaptic contacts between photoreceptors and neurons in the outer retina we are aiming to unveil the connectivity patterns between different rod types, and the pathways that might enable the observed 'ON-suppressed' output.

Session: Participant Symposium 4

Category: Vision and photoreception

Seahorse visual systems: Multiple regional specialisations within the retina support small prey capture

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Vision is central to feeding in many fishes, including seahorses. Seahorses hunt using pivot feeding, a highly effective strategy to identify, track and strike planktonic prey with incredible accuracy. This relies on advanced visual capabilities to identify small mobile prey against the background, resolve sufficient detail to confirm item suitability, maintain focus despite movement, and crucially, perceive depth allowing accurate strike. Seahorses achieve this using independently moving eyes with gross morphologies that optimise small prey detection. However, the visual system of seahorses at the retina level has not been studied in detail. Here, we present the results of our investigations into how the seahorse retina supports targeted prey strike.

In all five species studied we identified multiple retinal regions characterised by differences in morphology and gene expression. Histology and MRI scans revealed multiple foveae. Slicing and wholemount techniques confirmed changes in cell density throughout the retina and beyond the foveae. Transcriptome sequencing revealed the expression of three opsin genes in the retina: sws2 (blue), rh2a (green) and lws (red). Notably, fluorescent in situ hybridisation showed distinct differences in opsin gene expression and co-expression within the same cone photoreceptor cells across the dorsal-ventral and nasal-temporal axes, including expression of only one opsin per cone within the fovea and a ventronasal absence of lws. These differences demonstrate support for dichromatic, trichromatic, and potentially tetrachromatic retinal regions. To further investigate the role of retinal regionalisation in prey strike, we conducted feeding trials on Hippocampus whitei to determine the effect on foraging when ambient light matched the sensitivity of the foveal single cones. Our study presents one of the most complex fish visual systems to date, highlighting the benefit of multi-technique multi-species comparisons in visual ecology.

Session: Participant Symposium 4

Category: Vision and photoreception

Chicken-egg question of evolution of color vision and information theory

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Did color vision evolve as an adaptation to seeing colorful signals, such as bird plumage, or did these colorful signals evolve as an adaptation to pre-existing color vision systems? Information theory can give answer to this chicken-egg question. The quality of color vision systems can be characterized by the amount of information they convey about certain colorful objects. Alternatively, it can be characterized by the information capacity – the maximum amount of information the system can transmit. While the amount of information depends on the input signals, such as bird plumage, the information capacity does not depend on the input and is determined by ambient illumination. Therefore, if the spectral position and numbers of photoreceptors can be explained as an optimization of the information capacity, we can conclude that color vision system is adapted to seeing a wide variety of colors rather than certain colorful signals. Calculations show that, at high illumination levels, the information capacity is optimized when four spectral receptor types are spread uniformly along the spectrum and when their sensitivities are narrowed by retinal filters. This arrangement is similar to that of many birds, which have four spectral types of single cones equipped with colored oil droplets. Therefore, it is likely that bird plumage evolved as an adaptation to existing color vision of birds rather than bird color vision evolved as an adaptation to plumage colors. At lower illumination levels, the information capacity is optimized by removing retinal filters and by decreasing the number of spectral types of photoreceptors. Therefore, color vision of many species having three or two spectral receptor types can be explained as an adaptation to dim light conditions. In some animals, such as fireflies and some butterflies color vision probably coevolved with colorful signals. In these cases, sexual selection was an important driving force of evolution.

Session: Participant Symposium 4

Category: Vision and photoreception

Life in dim light: Visual development in nocturnal and deep-sea fishes

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Vertebrate vision is mediated by two types of photoreceptors, rods for dim light and cones for bright light, a combination of which is usually used in intermediate conditions. During development, the current dogma is that vertebrates start with a cone-dominated retina, with a single layer of rods added later. However, several non-conventional visual adaptations have been found in species from dim light environments, such as nocturnal reef fishes and deep-sea fishes. These include multiple rod layers (i.e., multibank retinas) and photoreceptors with a mixture of cone- and rod-like features (i.e., transmuted photoreceptors). Although these adaptations have been observed in adult fishes from distant lineages, little is known about how they develop or, in some cases, even what they do. To remedy this, I used transcriptomics, histology, and electrophysiology to study vision across development in >15 species of nocturnal reef and deep-sea fishes. I found that nocturnal coral reef fishes in the family Holocentridae add rod layers over ontogeny to obtain a densely packed rod-dominated retina with up to 17 distinct layers. Electroretinography suggested that these rod layers offer the sensory advantage of faster vision and enhanced light sensitivity. Furthermore, I found that several deep-sea fishes have evolved a pathway for visual development that is unique among vertebrates. As larvae, they have transmuted rod-like cone photoreceptors, whereas adults have pure rod retinas. Overall, these findings challenge our understanding of vertebrate visual development and enhance our understanding of the evolutionary and developmental dynamics of vision in extreme environments.

Session: Young Investigator Sympopsium 1

Category: Learning, memory and cognition

Connectomic mapping of navigational neural circuits in bees, ants and shrimps

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Arthropods constitute the most species-rich and arguably the most behaviourally diverse animal phylum on Earth. Despite this, the only arthropod species for which we have a comprehensive map of neurons and their connections, a ‘connectome’, is the fruit fly, Drosophila melanogaster. By serving as a framework for matching genetically accessible circuits to their homologous counterparts, the recently available public database of the fly connectome has already proved to be a remarkably powerful tool for gaining insights into circuit function. However, it also provokes a major question: How applicable is the fruit fly connectome when attempting to understand the neural control of behaviours in other insect species? To address this, we acquired a complete electron microscopy volume of an iconic associative memory centre in the insect brain known as the mushroom body (MB) in an adult army ant worker (Eciton hamatum). The three layer expand-converge architecture of the MB, also found in early models of the perceptron and in the cerebellum, is known to serve as a substrate for Pavlovian associative learning in insects. Army ants are eusocial insects that forage in swarms and are known to build bridges and nest structures using their bodies as structural components. The lifestyle of an army ant differs notably from that of a fruit fly, yet it is still relatively evolutionarily close within the arthropod phylum, making it an ideal candidate to probe the impact of behavioural diversity on seemingly conserved neural circuits. Furthermore, the army ant MB connectome will be a useful proxy to the clonal raider ants, which have recently emerged as the first ever transgenic ant model. By utilising a comparative approach, we are leveraging evolution as a powerful tool to gain a mechanistic understanding of neural circuits involved with the formation, storage, and retrieval of memories.

Session: Young Investigator Sympopsium 2

Category: Motor systems, sensorimotor integration, and behavior

The Aerial Dogfighting Strategy of Dragonflies

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Standing at the edge of a pond, we commonly see dragonflies tearing through the air in rapid engagements. These engagements, or pursuits, serve three functions: (1) predation, (2) territoriality, and (3) mating. A single male dragonfly must succeed in all these tasks to pass his genes on to the next generation, yet we know little about how dragonflies adapt their pursuit tactics to serve each function. Using high-speed videography in the field with a stereographic rig, we reconstructed the flight trajectories of dragonflies in predatory and conspecific engagements. Interactions between males are akin to the dogfights of fighter aircraft, in which either party can switch roles between chaser and evader. Dogfighting flight trajectories did not resemble those for prey interceptions, showing a fundamental switch in strategy. During predatory engagements, prey was always silhouetted against the sky with the hunting dragonfly shadowing behind the prey. In contrast, a territorial dragonfly could detect intruders from low visual angles against surrounding visual clutter and maintained these visual conditions during pursuit. Unlike predatory engagements, duelling male dragonflies did not intercept each other, using excess speed to fly circuitous loops around their opponent. Dogfighting dragonflies regularly switched roles from evader to a pursuer. In doing so, dragonflies used a repeated manoeuvre involved a sharp spiralling downward dive. We demonstrate that this structure, along with many of the flight patterns are emergent from a simple control strategy to get below and behind the target. During territorial conflicts, dragonflies show extreme manoeuvrability. Kinematic reconstructions used to identify the performance envelope demonstrate that dragonflies can sustain 6-G turns at high speed. Despite this high activity state, dragonflies glided for 36% of the time even whilst fighting, conserving energy whilst at high speed and throttling energetic input.

Neuroethological aspects of spatial navigation in toads and sleep in mice: a story of two tales

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As a neuroethologist who loves her research field, I have had a colorful background. In this presentation, I will be telling you two stories that summarize my journey through my PhD and my postdoc and (I hope) are meant to be inspiring. First, I will be speaking about my work in spatial navigation in an amphibian species, the common toad Rhinella arenarum. I will summarize studies where we determined that toads are successful navigators that can use plenty of sources of spatial information to find a reward in the environment. Briefly, we were able to train toads in spatial tasks employing different kinds of visual, auditory or self-centered cues and boundary geometry of the environment, and we found that all of these tasks were dependent on the hippocampal formation. Although at first sight this finding shares a striking resemblance with mammalian navigation, the story is far from being so simple, as mammals do not share a similar hippocampal organization and toads, on their end, do not have the strongly developed neocortex found in mammals. Our work has unveiled new avenues to explore in the field of spatial navigation in a key vertebrate species that directly descends from the first tetrapods that colonized earth.

Second, I will be moving on to my postdoctoral work where I focused on sleep research in rodents, employing freely moving neurophysiological toolkit and video monitoring. Our work revealed that mice, such as many other animals, display a characteristic behavioral repertoire before going to sleep, that includes nest-building and grooming behavior. Moreover, combining cell-specific manipulations we found that nest-preparation before sleep is in part guided by a glutamatergic neuronal population in the lateral hypothalamus. In another part of this sleep research journey, we found that mice prepare to sleep and sleep while huddling with their conspecifics while housed in a social context, and that brain oscillations and vigilance stages are synchronized among them. This work during my postdoc has shed light on the importance of the sleep preparatory routine and the environmental context on sleep physiology and plasticity.

Session: Young Investigator Sympopsium 4

Category: Spatial orientation and navigation

Multiple compass cues guide Monarch butterflies during migration

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Each fall millions of Monarch butterflies (Danaus plexippus) migrate over thousands of kilometers from North America to their overwintering habitat in Central Mexico. To maintain their southerly direction, these butterflies rely on the sky for orientation. But which celestial cues they use exactly and which of them are essential to set the migratory direction is still unclear. To investigate this, we tested migratory Monarch butterflies while they were tethered at the center of a flight simulator and were able to freely change their bearing. When we tested the butterflies in this simulator outdoors, they kept constant southward directions under the natural sky. When we displaced the sun by 180° using a mirror, most animals changed their heading direction by about 180°. This suggests that the sun acts as their main migratory cue. We next tested the butterflies under a linear polarization filter (while shading the sun) and found that the migratory animals turned their heading direction when the polarizer was rotated by 90°. Thus, in the absence of the sun, the butterflies use the polarization pattern of the sky for orientation. We then investigated if the butterflies were able to keep their migratory direction in the absence of all skylight cues and found that, even without any skylight cue, their headings remained directed into the corrected migratory direction. Taken together, our findings show that multiple skylight cues guide Monarch butterflies on their long journey to Central Mexico. Even without skylight cues, the butterflies kept their migratory route, suggesting that they rely on additional cues for migration. We propose that Monarch butterflies fall back on a non-visual cue, such as the geomagnetic field, to maintain their migratory direction even if no reliable visual cues are available.

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Session: Plenary 4

Motor control of facial expressions in non-human primates

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Humans and non-human primates communicate their emotions through facial expressions. An extensive set of motor cortical areas in the frontal lobe are devoted to controlling the facial musculature through direct projections to the facial nucleus. The classic view of the system holds that the challenge of controlling different types of facial movements is simplified by functional segregation where a medial system is dedicated to controlling emotional expressions, and a lateral one for voluntary control over facial movements. However, very little is known about the way this distributed network produces coherent and meaningful motor output.

To address this question, we combined MRI-targeted electrophysiology, recording of neural activity and electrical stimulation when monkeys perform voluntary and emotional facial movements. Ethologically meaningful facial expressions were elicited using a combination of dynamic visual stimuli, an interactive avatar and real-life interactions with conspecifics or experimenters.

Neural activity in all cortical areas exhibited overlapping, mixed representations of external cues, inner states, and motor commands, contrary to the expected medial-to-lateral functional dichotomy. Despite this tangled organization of the neural activity, the output effects of face-related cortical sites were conserved with no clear evidence of an underlying action map. Among the face-related cortical areas, the ventral premotor cortex appeared to function as a hub for coordinating state-specific facial movements.

These findings suggest that motor control over facial expressions is achieved through an interplay between inter-areal communication and local dynamics which can distil the multiple (often conflicting) signals into unambiguous motor output. In the absence of action maps, these interactions, together with converging subcortical signals, facilitate the production of coherent facial movements.

Session: Invited Symposium 5

Category: Olfaction, taste and chemical sensing

Functional evolution of the taste and digestive system in birds

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Animals inhabit very different environments and need to find and utilize very different sources of nutrition. As a result, sensory and digestive systems can vary tremendously across species. Examining these sensory and physiological changes across diverse species in a comparative context yields insight into the evolution of the nervous system and animal behavior, and into broad questions about the evolutionary process, the evolution of novel protein functions, and coevolution between different organismal systems. For example, birds are ancestrally sweet-blind, as they have lost the canonical taste receptor (T1R2) used by mammals to detect sugar. Multiple lineages--especially those that consume nectar or large amounts of fruit--have regained the ability to sense carbohydrates using the savory receptor (T1R1-T1R3), and these sensory shifts are also often accompanied by metabolic and digestive changes. Here, I discuss our work unraveling the history of sugar detection across the bird phylogeny, and examine the interplay of functional shifts in digestive enzymes with changes in taste receptors to probe how sensory systems evolve and how organismal physiologies can shift in response to changes in diet.

Session: Invited Symposium 5

Category: Other

Cellular and molecular adaptations to acute mechanosensitivity in the bill of tactile foraging birds

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Symposium 5 Presenter "Rebalancing diversity of animal models in neurophysiology and sensory biology"

Tactile specialist birds of the Anatidae family can efficiently select edible matter in muddy water, relying on the sense of touch in their bill, and thus provide a trackable model to understand cellular and molecular principles of mechanosensory plasticity. Efficient foraging is enabled by Meissner (Grandry) and Pacinian (Herbst) corpuscles, which detect transient touch and vibration, and are present in bill skin at densities higher than in the human hand. We show that to support the high-density population of corpuscles, mechanoreceptor neurons in trigeminal ganglia undergo numerical expansion at the expense of pain- and temperature receptors, and this trend correlates with tactile specialization across a panel of bird species. Upon reaching the skin, mechanoreceptor terminals interact with Schwann cell-derived lamellar cells (LC) to form the sensory core of corpuscles. Using enhanced focused ion beam scanning electron microscopy, we revealed the 3D architecture and ultrastructural relationship between LCs and mechanoreceptor terminals. Direct ex vivo electrophysiological recordings form corpuscles showed that LCs are mechanosensitive and excitable, serving as physiological sensors of touch. The elaborate architecture and bi-cellular sensory mechanism in the corpuscles, which comprises the nerve terminal and LCs, create the capacity for nuanced encoding of the submodalities of touch during foraging. This work is supported by grants from NSF (1923127) and NIH (1R01NS097547, R01NS126277).

Session: Invited Symposium 5

Category: Olfaction, taste and chemical sensing

Neuropeptide Regulation of Mosquito Host-seeking Behavior

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We to use genetic and pharmacological approaches to identify the genes, signaling pathways, and circuits that regulate host-seeking behaviors in Aedes aegypti mosquitoes.

Hardwired circuits provide a structure for behavior, for example by encoding responses to sensory cues emitted by human hosts, and neuromodulators shape and specify when and how innate behaviors are performed. The drive to find and bite humans is not constitutive, but is regulated by the female mosquito's internal state as well as environmental cues. Using pharmacological and genetic approaches we have identified neuropeptide signaling pathways that regulate host-seeking behavior after a blood meal and by time of day.

Aedes aegypti females normally suppress their host-seeking drive after a complete blood meal, while eggs are developing. We have identified a hindgut-expressed Neuropeptide Y-like receptor (NPYLR7) that coordinates host-seeking suppression, egg development, and nutrient utilization after a blood meal. Pharmacological or genetic disruption of this pathway leads to inappropriate attraction to human host cues, while exogenous activation of this pathway suppresses host attraction even in the absence of a nutritive blood meal.

The temporal organization of biting activity varies between mosquito species and Aedes aegypti show the highest levels of biting near dawn and dusk. We show that Aedes aegypti change their behavioral responses to CO2, a potent host-associated chemosensory cue, at different times of the day. The circadian neuropeptide Pigment Dispersing Factor (PDF) is a critical regulator of daily activity and disrupting PDF signaling alters the temporal organization of daily activity and host cue responsiveness.

Beyond the significance of this work to basic neuroscience discovery, these behaviors play key roles in vector biology. Mosquito-borne diseases pose increasing threats to global public health and our work can provide new targets to prevent mosquito/host interactions.

Session: Invited Symposium 5

Category: Metabolism, biological rhythms and homeostasis

Neuroendocrine responses to long-term water deprivation in a mammalian hibernator

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While most mammals cannot survive for more than a few days in a state of dehydration, the thirteen-lined ground squirrel (Ictidomys tridecemlineatus) routinely hibernates for up to 8 months of the year without access to water. We recently showed that hibernating squirrels remain hydrated in the face of extreme water deprivation by differentially regulating extracellular concentrations of electrolytes to significantly decrease blood osmolality during torpor. Furthermore, in a transient period of arousal during hibernation called interbout arousal, serum osmolality and plasma levels of the antidiuretic hormones arginine-vasopressin (AVP) and oxytocin (OXT) increase dramatically, yet drinking behavior is completely suppressed. In mammalian non-hibernators, osmolality, AVP and OXT levels and drinking behavior are inextricably linked; the mechanism by which these parameters can be decoupled is unknown. Here, we demonstrate that the AVP/OXT release pathway is activated by hypothalamic supraoptic nucleus (SON) neurons early in the transition from torpor to interbout arousal. SON neuron activity, dense-core vesicle release from the posterior pituitary, and plasma hormone levels all begin to increase before the animal's brain and body temperatures reach 10°C. Simultaneously, thirst suppression arises from neurons in the circumventricular organs (CVO) which are responsible for sensing and responding to thirst stimuli. Electrophysiological and circuit-level alterations to neural function allow CVO neurons to enforce thirst suppression despite endocrine signals indicative of profound dehydration. Our work reveals the extent to which the mammalian neuroendocrine thirst pathway can be adapted, and paves the way for future studies of survival in the face of extreme environmental challenges.

Session: Invited Symposium 6

Category: Spatial orientation and navigation

Neural computations during naturalistic spatial behaviors in bats

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---Talk from the symposium ‘Finding food, home and friends: neuroethology of navigation across species and behaviors’---

Understanding how animals – individuals as well as groups – navigate in their natural environments is crucial for uncovering the neural computations underlying spatial behaviors. While much research has been done on how our brains navigate in static environments, the neural basis of navigation in dynamic naturalistic environments remains less well understood. To address this gap, we studied neural activity in the hippocampus of Egyptian fruit bats using a variety of methodologies (single unit and population recordings), and under a range of naturalistic behavioral conditions. I will highlight some key findings that were reached through this neuroethological perspective. This will demonstrate the significance of naturalistic approaches combined with cutting-edge techniques for recording neural activity and behavior in freely moving (and flying) animals. Through this approach, we shed light on the mammalian neural computations involved in spatial behaviors, providing valuable insights into how animals may represent and navigate their dynamic environments.

Session: Invited Symposium 6

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Integrating neural modeling, robotics, and behavior to study magnetoreception

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After decades of successful research, in many ways the magnetic sense remains an outstanding mystery in the field of sensory biology. We know that the sense is phylogenetically widespread and provides many species with both directional and positional information. However, no receptor has been conclusively identified in any animal and as such, the physiological function, limits, and processing of this sense are largely unknown. Here, we present an approach developed to systematically study the magnetic sense in this context. We develop narrow hypotheses for how magnetic sense in this context. We develop narrow hypotheses for how magnetic transduction and processing may be structured based on species-specific neural architecture and prior magnetoreception research. Then, we compare the performance of animals in field behavioral assays to the performance of our hypotheses in neural computational and robotic models performing the same assay. In testing the predictive power of our hypotheses across multiple behavioral measures using both computational and robotic models, we are able to assess the likelihood that any given hypothesis begins to describe how this enigmatic sense may function. We demonstrate this approach in the Caribbean spiny lobster Panulirus argus, which shows remarkable abilities to navigate using Earth's magnetic field. In doing so, we eliminate some possibilities of how the sense may function in P. argus and bolster others. After this efficacy in studying magnetoreception in lobsters, we believe our approach may prove a promising tool for understanding magnetoreception more broadly.

Session: Invited Symposium 6

Category: Spatial orientation and navigation

GPS-tracking of pigeons while homing reveal a broader functional profile of the avian hippocampus: Visual-spatial attention/perception

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The behavioral and neural mechanisms that support goal-directed, spatial navigation have been an enduring interest of neuroethologists. One manifestation of this interest, inspired by the idea of studying spatial navigation under natural field conditions, has been field and laboratory research, including the single unit, neuronal recordings, carried out to understand the role of the avian hippocampal formation (HF) in supporting the homing behavior of pigeons. Emerging from that research has been a functional profile of HF that aligns well with the canonical narrative of a hippocampus important for spatial memory and the implementation of such memories to support navigation. However, recently an accumulation of disparate observations has prompted a rethinking of the avian HF as a structure also important for the control of visual-spatial perception or attention antecedent to any memory processing. I will summarize field observations contrasting the behavior of intact and HF-lesioned homing pigeons from several studies, based primarily on GPS-recorded flight paths, that support a recharacterization of HF's functional profile to include visual-spatial perception.

Session: Invited Symposium 6

Category: Learning, memory and cognition

Coding of episodic memories in the hippocampus of a food-caching bird

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Throughout each day the brain captures snapshots of distinct experiences, forming episodic memories that often last a lifetime. This function depends on the hippocampus – a brain region that is evolutionarily conserved across vertebrates. My lab studies the relationship between hippocampal activity and episodic memory using a unique model organism – the black-capped chickadee. Chickadees are specialist food-caching birds that store thousands of food items at concealed locations in their environment and use memory later in time to retrieve their caches. I will describe our effort in designing behavioral arenas and neural recording techniques to study these behaviors in laboratory conditions. I will share our discoveries of spatial representations in the chickadee hippocampus, as well as our latest data on how neural activity in this region represents the content of distinct memories. Finally, I will offer a general, mechanistic model of episodic memory in the hippocampus inspired by our chickadee data.

Session: Invited Symposium 7

Category: Motor systems, sensorimotor integration, and behavior

Is love blind? Mating proximity gates threat perception.

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When forced to choose between fundamental needs, making the wrong decision could prove fatal. However, it is currently unclear how alternative options are evaluated and appropriate actions are prioritised. To tackle this problem, we developed an experimental system to study the neural circuit mechanisms that integrate the benefit of imminent courtship success with the risk of predation in Drosophila. By combining our novel behavioural assay with neurogenetics, connectomics and live imaging, we identified the neural circuitry that establishes behavioural priority during this 'life-death' conflict. Crucially, we found that the probability of mating success defines the decision to reproduce or flee. Our work reveals how the brain weighs up antagonistic advantages and risks, and the probability of success, at a cellular-circuit level.

Session: Invited Symposium 7

Category: Other

Mapping Neural Circuits of State-dependent Behavior in the Fly

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Perceptions and decisions depend on sensory impressions, but also on past experiences and the present internal state of an animal. Behavior is therefore very adaptive and flexible. For instance, a hungry animal perceives the smell and taste of food as much more positive than a fed animal. At the same time, it is willing to take a high risk and invest time and energy in order to find food. Which signals and neural networks allow the communication between brain and body? And how do they modulate behavior and decision-making in the best interest of the organism?

We aim at answering these questions at three levels: (1) behavior, (2) neural networks, and (3) genes. To this end, we are using Drosophila genetics in combination with modern techniques including high resolution behavioral analysis, optogenetics, and in vivo whole brain and multiphoton microscopy. In particular, we focus on how the brain dynamically translates chemosensory information, i.e. odors and tastes, into state- and experience-dependent perceptions and ultimately into behavior. In my talk, I will discuss two recent examples of our ongoing work.

Session: Invited Symposium 7

Category: Vision and photoreception

Vision for predator evasion and predation

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I will discuss ongoing work from my group on the visual pathways that (1) detect approaching predators and drive evasive actions and (2) detect prey and guide pursuit and capture. We combine genetic and viral engineering, electrophysiology, functional and ultrastructural imaging, quantitative behavioral analyses, and computational approaches to delineate these cell-type-specific pathways and understand their visual computations. We also, compare the hunting behavior of mice to a mouse-sized marsupial predator, the fat-tailed dunnart.

Session: Invited Symposium 7

Category: Other

A theory of rapid behavioral inferences under the pressure of time

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To survive, organisms must make rapid inferences about their surroundings. For example, to successfully escape an approaching predator, animals must estimate the direction of approach from noisy stimuli. Such rapid inferences are particularly challenging, because the organism has only a brief time window to gather sensory information, yet the precision of inference is critical for survival. Due to evolutionary pressures, nervous systems likely evolved effective computational strategies that enable accurate inferences under strong time limitations. Here, we aim to understand the principles underlying such rapid inferences. We consider a model scenario in which an observer has to determine the direction of an approaching predator from a brief sequence of noisy sensory signals. Traditionally, the relationship between the duration of inference and its accuracy has been described as a ``speed-accuracy tradeoff" (SAT). Intuitively, the longer the observer collects sensory information, the more accurate the inference that can be made from that information. We focus on scenarios where the observer is faced with strong time constraints, and thus cannot perform accurate inference. Our key insight is that while the SAT characterizes performance on average, it can be decomposed into diverse patterns of error dynamics on individual trials. Each pattern is generated by a different stimulus sequence that occurs by chance. By computing information-theoretic quantities, we were able to differentiate ``optimistic" and ``pessimistic" stimulus sequences. Optimistic sequences permit rapid and accurate inferences, while pessimistic sequences generate high errors that persist until the time limit. To exploit this single-trial structure, we derived adaptive stopping rules that rely on the dynamics of posterior uncertainty. These rules (i) increase both the speed and accuracy of inferences, (ii) violate the SAT, and (iii) qualitatively reproduce features of escape behaviors in insects.

Session: Invited Symposium 8

Category: Vision and photoreception

Colour vision in fishes: an investigation of discrimination thresholds through colour space

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Colour vision guides important behaviours, including foraging, predator avoidance and communication. The ability to discriminate between similar colours is limited by noise in photoreceptors and subsequent neural processing. Theoretical models based on sensory input and visual system parameters are frequently used to estimate visual performance; however, few studies have undertaken comprehensive tests of colour discrimination in non-human species to compare model predictions with behavioural data. Here, we present studies on a non-UV sensitive fish (triggerfish Rhineacanthus aculeatus) and a UV-sensitive anemonefish (Amphiprion ocellaris). We first investigated the anatomical and molecular basis of their visual systems using microspectrophotometry, opsin gene expression, fluorescence in situ hybridisation (FISH) and retinal wholemounts. Next, using behavioural trials, we measured behavioural thresholds by training fish and assessing their ability to distinguish an odd-coloured target from distractors of varying intensity based on Ishihara-style stimulus patterns. In triggerfish, colour discrimination thresholds for 21 directions at five locations in color space matched model predictions most closely for stimuli near to the achromatic point, but exceeded predictions (indicating a decline in sensitivity) with distance from this point. Thresholds were higher for saturation than for hue differences. For anemonefish, UV colour discrimination for nine sets of UV and non-UV colors were tested using a novel five-channel (RGB-V-UV) LED display. Discrimination thresholds were consistently lower (i.e., more-acute) for targets with UV-contrast compared to those without. UV as a component of colour therefore make stimuli more salient and likely improves the detectability of UV body patterns viewed by a reef fish. These changes in color thresholds with color space location and direction may give insight into photoreceptor non-linearities and post-receptoral mechanisms.

Session: Invited Symposium 8

Category: Vision and photoreception

Ancestral photoreceptor diversity as the basis of visual behaviour

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Animal colour vision is based on comparing signals from different photoreceptors. It is generally assumed that processing different spectral types of photoreceptor mainly serves colour vision. Here I propose instead that photoreceptors are parallel feature channels that differentially support visual-motor programmes like motion vision behaviours, prey capture and predator evasion. Colour vision may have emerged as a secondary benefit of these circuits, which originally helped aquatic vertebrates to visually navigate and segment their underwater world. Specifically, I suggest that ancestral vertebrate vision was built around three main systems, including a high-resolution general purpose greyscale system based on ancestral red cones and rods to mediate visual body stabilization and navigation, a high-sensitivity specialized foreground system based on ancestral ultraviolet cones to mediate threat detection and prey capture, and a net-suppressive system based on ancestral green and blue cones for regulating red/rod and ultraviolet circuits. This ancestral strategy probably still underpins vision today, and different vertebrate lineages have since adapted their original photoreceptor circuits to suit their diverse visual ecologies.

Session: Invited Symposium 8

Category: Spatial orientation and navigation

Mechanisms of spectral orientation in dung beetles

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The diurnal ball-rolling dung beetle Kheper lamarcki uses a large range of celestial cues to steer straight across the savannah, but it is not known how they extract directional information from the spectral gradient that spans the sky. This gradient, which arises due to the scattering of sunlight, allows for a discrimination of the solar half of the sky—that is richer in longer wavelengths of light—from the anti-solar half of the sky. In this study, we define the spectral sensitivity of the diurnal dung beetle K. lamarcki and use the information to explore the orientation performance under a range of spectral light combinations. Our results reveal that when presented with spectrally diverse stimuli, the beetles primarily orient to the apparent brightness differences as perceived by their green photoreceptors. Under certain wavelength combinations, they also rely on spectral information to guide their movements, but the brightness and spectral directional information is never fully disentangled. Overall, our results suggest the use of a dichromatic, primitive colour vision system for the extraction of directional information from the celestial spectral gradient to support straight-line orientation.

Note: This is an invited talk and will be given at the Symposium on Mechanisms of Color Vision: Genes, Eyes, Neuronal Circuits, and Behavior.

Session: Invited Symposium 8

Category: Vision and photoreception

Sex-linked gene traffic underlies the acquisition of sexually dimorphic UV color vision in Heliconius butterflies

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The acquisition of novel sexually dimorphic traits poses an evolutionary puzzle: How do new traits arise and become sex-limited? Recently acquired color vision, sexually dimorphic in animals like primates and butterflies, presents a compelling model for understanding how traits become sex-biased. For example, some Heliconius butterflies uniquely possess UV (ultraviolet) color vision, which correlates with the expression of two differentially tuned UV-sensitive rhodopsins, UVRh1 and UVRh2. To discover how such traits become sexually dimorphic, we studied Heliconius charithonia, which exhibits female-specific UVRh1 expression. We demonstrate that females, but not males, discriminate different UV wavelengths. Through whole-genome shotgun sequencing and assembly of the H. charithonia genome, we discovered that UVRh1 is present on the W chromosome, making it obligately female-specific. By knocking out UVRh1, we show that UVRh1 protein expression is absent in mutant female eye tissue, as in wild-type male eyes. A PCR survey of UVRh1 sex-linkage across the genus shows that species with female-specific UVRh1 expression lack UVRh1 gDNA in males. Thus, acquisition of sex linkage is sufficient to achieve female-specific expression of UVRh1, though this does not preclude other mechanisms, like cis-regulatory evolution from also contributing. Moreover, both this event, and mutations leading to differential UV opsin sensitivity, occurred early in the history of Heliconius. These results suggest a path for acquiring sexual dimorphism distinct from existing mechanistic models. We propose a model where gene traffic to heterosomes (the W or the Y) genetically partitions a trait by sex before a phenotype shifts (spectral tuning of UV sensitivity).

Session: Plenary 5

The evolution of ant social behavior

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The colonies of social insects are complex biological systems in which manifold interactions between individuals give rise to emergent properties that are adaptive at the group level. However, most social insects cannot be propagated in captivity or genetically manipulated, severely limiting the scope for experimentation. Over the past twelve years, we have developed and utilized the clonal raider ant, Ooceraea biroi, as a new model species that overcomes many of these limitations, allowing us to study social dynamics and underlying mechanisms under controlled laboratory conditions. Our work has led to a deeper understanding of how ants within a colony assume distinct behavioral roles and efficiently divide labor, how they communicate, both as adults and across different developmental stages, and how these interactions result in collective behavior. Additionally, our research has shed light on how evolution has repurposed and expanded genetic, neural, and physiological mechanisms from solitary ancestors to produce highly social organisms. In this seminar, I will provide an overview of this work and discuss future directions in our research program aimed at understanding the evolution and organization of insect societies, spanning from genes to neural circuits and behavior.

Oral Communications
Thursday, 01 August 2024

Machine intelligence inspired by nature: From locomotion to manipulation and navigation

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Living creatures, like walking animals, can rapidly develop their gaits shortly after birth, thanks to their neural control circuits which are genetically encoded. The neural circuits also enable them to adapt their movements to traverse various substrates, deal with damage, and even take proactive steps to avoid colliding with an obstacle. In addition to adaptive locomotion, they can also perform diverse complex autonomous behaviors, such as object manipulation/transportation, navigation, and their combination, all achieved with a remarkable level of energy efficiency. Biological studies reveal that these complex capabilities are largely achieved not only through neural control circuits but also through several ingredients, including sensory feedback and dynamic embodied interactions across the body, brain, and environment. Additionally, their biomechanics (morphological computation) also plays a crucial role in facilitating robust behavior. In this talk, I will discuss and demonstrate "how we can realize these ingredients with embodied intelligence inspired by nature for complex machines (robots) so they can show motion intelligence like their biological counterparts".

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

The Neural Basis of Parent-Offspring Communication in Poison Frogs

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Establishing a strong social bond with our caregivers lays the foundation for offspring survival and fitness. As offspring often cannot regulate their nutrition, signals of need require accurate and precise interpretation by their caregivers to provide care. However, little is known about the neural basis of communication between parents and offspring. Poison frog tadpoles are altricial and rely entirely on parental investment for healthy development. In the Mimic poison frog (Ranitomeya imitator), mothers provide more food to tadpoles that beg (vibrate) more intensely, suggesting somatosensation (touch) is an important component of offspring signaling need. To test this hypothesis, we created a robotic tadpole with modifiable characteristics to emulate offspring signals, along with a customized camera trap that is specifically designed for ectotherms. We found that parents, depending on sex, may use different offspring signals to make care decisions. In parallel, we explored how parental brains respond to the begging behavior of their offspring. We found that offspring touch coincides with activation of opioid pathways and suppression of nociceptive pathways. When opioid signaling is perturbed, we found that fathers modify their contact with offspring, suggesting an evolutionarily ancient role for these neural pathways in parent-offspring bonds on a somatosensory and behavioral scale. Using these two advances, we are beginning to decipher which infant communication components contribute most to a parent's choice to nurture or to neglect.

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

Evolution of Predatory Behaviour and Aggression in Nematodes via Noradrenergic-Like Circuits

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Within the nematode clade, a huge variety of different behavioural strategies have evolved in accordance with the different ecologies and environments associated across the diverse species. Pristionchus pacificus, while similar to Caenorhabditis elegans in many regards, has acquired several distinctive features and additional behaviours. Specifically, like C. elegans, P. pacificus can feed on bacteria, but its foraging behaviour can also encompass the predation of other nematodes.

Prior studies showed that the rate of pharyngeal contractions strongly differs whether the animal is feeding on larvae versus bacteria. To study the regulation of feeding further, we developed an automated behavioural tracking system and machine learning model to cluster and predict persistent and robust behavioural states in P. pacificus. We present a semi-supervised machine learning model that can reliably predict feeding behaviours in P. pacificus from tracking data, acquired by PharaGlow, a tool for tracking and analysing locomotion and feeding behaviours of moving worms from video data. Our model shows how animals transition between predatory and non-predatory feeding states, and how posture, motion and feeding differ between the different feeding states. Using these newly developed methods, we screened mutants defective for the major biogenic amines in nematodes and found that octopamine and tyramine antagonistically regulate predatory versus non-predatory feeding states. We find that octopamine induces a predatory state and is dependent on the Ppa-ser-3 and Ppa-ser-6 receptors, whereas tyramine opposes predatory behaviour via the ligand-gated ion channel lgc-55. Furthermore, ongoing gene expression analysis suggests evolutionary divergence in the localisation of these receptors between C. elegans and P. pacificus.

In conclusion, we have identified aggressive behaviours and its underlying regulatory circuit in nematodes associated with the development of predatory behaviour.

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

A novel framework for social learning in Drosophila

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Mammals, birds, and insects learn from social experience to increase their social status, reproductive success, and chances of survival. Inability to learn from social experience can severely impair life in a social world. Yet, we do not fully understand how social experience shapes behavioral strategies through learning, because in most systems we lack i) tools to perturb social interactions, and ii) a general framework to measure behavioral strategies and their dependence on social experience. We aimed at closing both gaps: we first developed an analytical framework that quantitates social experience and behavioral strategies by decomposing data from automated pose estimation in freely behaving animals into a set of base experiences and base strategies. We applied this framework to show that the innate courtship strategy of male Drosophila is shaped by past social experience. Specifically, we developed an assay for social learning that comprised two experiments: in a first training experiment, closed-loop optogenetics controlled female feedback to male courtship song, to systematically perturb male social experience around the time of song. In a subsequent test experiment, we applied our analytical framework to evaluate the trained males' song strategies towards females providing unperturbed feedback. Compared to controls, males that experienced perturbed feedback during training used distinct song strategies during testing, suggesting the innate courtship strategy can be shaped by learning from social experience. Known learning mutants and males with genetically downregulated dopamine receptor expression lacked social learning in our assay, corroborating our findings. These results demonstrate a surprising flexibility of fly courtship behavior, and open the door for a circuit-level understanding of this type of social learning. Our analytical framework is applicable in any system to quantitate behavioral strategies and their dependence on social experience.

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

Parental early social experiences have neurodevelopmental effects on the mesolimbic reward system in the offspring

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Early-life experiences can have long-term effects on the adults' phenotype, but the degree to which these effects carry over to future generations is largely unknown. In the cooperatively breeding cichlid Neolamprologus pulcher, early social experiences determine the offspring's ability to adjust their response to the social information, aka social competence. Previous work shows the relative importance of the dopaminergic system in regulating social interactions and that early social experiences lead to life-long changes in the expression of dopamine receptors within the social decision-making network (SDMN). Here we asked if parental early social experience and/or own early social experience leads to changes in the expression of dopaminergic receptors in two regions from the SDMN which also belong to the mesolimbic reward system. In a 2x2 full-factorial design, we generated F0 breeder pairs that had been reared either with (+) or without (-) parents in early life. This resulted in four lines, in which F1 fish either experienced the same (+/+ and -/-) or the opposite social environment as their parents (+/- and -/+). After the F1 reached adulthood, we micro-dissected the putative homologous regions to the medial Amygdala (Ventral pallium, Vs) and the nucleus accumbens (ventral dorsal telencephalon, Vd), and quantifying the expression of drd1 and drd2 receptors. We show that parental early social experience affects the constitutive expression of drd1 and drd2 expression in the Vd. While F1's own early social experience does not influence the constitutive expression of drd1 and drd2 in the Vd nor the Vs. Our results highlight the importance of parental early social experience on the neurodevelopment of their offspring.

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

Exploring interactions between the circadian clock and memory centers in honey bees

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Honey bees show a high degree of plasticity in the timing of daily foraging activities and a remarkable capability to form multiple time memories. These capabilities are likely adaptations to optimize the energy efficiency of social foraging. Given the multitude of behavioral evidence for a close connection between the circadian clock and learning and memory processes, we chose honey bees to explore molecular connections between the clock and the mushroom bodies. Based on earlier experiments which indicated that the small Kenyon cells (sKC) are likely involved in time-memory processes, we performed a detailed time-series RNA-seq analysis and single molecule fluorescent in situ hybridization study (smFISH) of the mushroom bodies and central complex of time-trained foragers (Roy et al 2024). These studies showed that most of the mushroom body Kenyon cells (KC) receive direct modulatory input from the central pacemaker via pigment dispersing factor. In addition, some of the KCs co-express cry2 and period suggesting that these KCs might even function as local clocks. The RNA-seq study on sKC-enriched tissue samples revealed that at least 862 genes show max or min peak expression at the time of training. Among these genes were 10 transcription factors (e.g. Egr1, Hr38, Creb, AP1), and several genes of known molecular pathways involved in learning and memory (e.g. octopamine and glutamate signaling, cAMP-PKA cascade). Finally, smFISH using the neuronal activity regulated transcription factor Egr1 showed that the sKCs get activated in advance of the training time suggesting that they might be involved in reactivation of memory or preparation of expected learning processes. The temporal organization of social activities makes honey bees a promising group to explore how the clock regulates time-memory and cognitive processes.

Roy et al. (2024) Multiple molecular links between the circadian clock and memory centers in honey bees. bioRxiv 2024.03.31.587450

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

Parasites exploit plasticity to reprogram the brain of social wasps

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While parasitic control of life-history traits is well-understood across many host-parasite systems, we still know little about how parasites manipulate the brain. Social wasp brains represent prime targets for parasites by providing the opportunity to exploit hosts that retain the ability to switch castes and behavior. Here, we take advantage of Polistes fuscatus wasps infected by twisted-wing insects to explore manipulation of neuroplasticity. Intriguingly, this parasite can inhibit reproduction, increase the lifespan, and manipulate social behavior in female but not in male hosts. Therefore, we predicted that the parasite could control social caste-related plasticity that mediates physiological, and behavioral traits critical for parasite survival and fitness. We compared neurogenomic signatures of infected and uninfected female workers, gynes (future foundresses), along with infected and uninfected male wasps. Transcriptomic analyses indicate that infected worker brains show similar signatures to uninfected gyne brains, highlighting genes shown to regulate longevity, reproduction, diapause, and neuromodulation. Proteomic comparisons confirm that parasites can exploit caste-related brain plasticity to modify behavior, physiology, and metabolism of female hosts. Together, our results reveal novel insights into understanding neuro-manipulation of social insects.

Session: Participant Symposium 5

Category: Social behavior and neuromodulation

Steroid modulation of aggressive behavior in Gymnotus omarorum: a seasonal perspective

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Sex steroids are key hormones in the modulation of aggressive behavior. Since most studies have primarily focused on species in which only breeding males express aggression, a better understanding of the hormonal mechanisms underlying this behavior can be reached by incorporating models which display aggression uncoupled from breeding and female aggression. The weakly electric fish, Gymnotus omarorum, is a seasonal breeder that expresses year-round aggression in both sexes. Previous studies using estrogen synthesis inhibitors have shown that non-breeding aggression is dependent on estrogens in both sexes, and gonads were ruled out as an estrogen source. Breeding aggression in G. omarorum, on the other hand, has not been studied. We conducted aggressive encounters during the breeding season. Dyads were treated with an estrogen synthesis blocker 30 minutes prior to interaction. We showed that estrogens were involved in female motivation to fight and male post-resolution aggression. We analyzed the expression of 3 steroid-related genes in 3 areas of the social brain and the circulating levels of steroids in the same individuals across sex and seasons. In both sexes during the non-breeding season brain aromatase levels were higher, circulating androgens were detectable, and circulating estrogens were undetectable. We propose that during non-breeding aggression, estrogens are synthesized locally in the brain from peripheral androgens. During the breeding season, brain aromatase was detectable in both sexes and females presented high levels of circulating estrogens, which might also modulate their aggressive behavior. In males, other mechanisms might be involved. In summary, the results show that estrogens are involved in aggression year-round. During the non-breeding season, estrogens are brain-derived, probably from circulating androgen precursors. In contrast, during the breeding season, there is sexual dimorphism both in source and effects of estrogens on aggression.

Session: Participant Symposium 5

Category: Other

Flight dynamic of swarming malaria mosquitoes

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With the increasing resistance of wild mosquitoes to insecticides, finding efficient alternatives to control these disease vectors is becoming increasingly urgent. One promising method is the sterile insect technique (SIT), which relies on the sexual competitiveness of lab-reared sterile males released into the field. However, to assess the potential of this vector control technique, we need a better understanding of mosquito mating behaviour.

Malaria mosquitoes typically gather in swarms at sunset to find mates. Initially, male mosquitoes congregate above a swarm marker, often a visually contrasting object on the ground. After a few minutes, female mosquitoes begin to approach the swarm, forming mating copulas with males, which then leave the swarm. However, our understanding of swarm dynamics and individual interactions remain limited. In particular, it is unclear how mosquitoes use swarm markers to coordinate their swarming behaviour.

To address this gap, we utilized stereoscopic videography-based tracking to reconstruct the three-dimensional flight kinematics of male malaria mosquitoes (Anopheles coluzzii) swarming in the laboratory. Using this data, we quantified the spatial and temporal dynamics of individual mosquito flight behaviour within the swarm, as well as their interactions with each other. Based on our observations, we propose a new set of behavioural rules for swarming mosquitoes that can account for most of the observed flight dynamics.

Session: Participant Symposium 6

Category: Motor systems, sensorimotor integration, and behavior

How spiders actively modulate web-vibration sensing during prey capture

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Organisms flexibly adjust postures to acquire information from environments based on real-time sensory feedback. This study investigates how spiders actively engage in a series of sensorimotor actions to manipulate the vibrational sensory field during prey localization. We hypothesize that orb-weaving spiders, Uloborus diversus, actively adjust leg posture and produce web vibrations to increase sensory gain.

To monitor both sensory inputs induced by prey, Drosophila, and the behavior of spiders, we used a high-speed camera to capture web vibrations, while side cameras recorded spider behavior simultaneously. We found that Drosophila produces low frequencies between 5-30 Hz on the web. During prey localization, spiders actively crouch and pull threads, inducing multiple harmonics on the web. By investigating sensory inputs from the prey and using the hidden Markov model, we can predict the spider's 3 behavioral outputs with 70% accuracy. Additionally, we used a piezo actuator to simulate prey signals with well-defined frequencies and amplitudes. This enabled us to deceive the spiders and analyze how leg posture is altered as a function of web frequency.

We also used scanning electron microscopy to examine the structure of the spider's vibration receptors, slit sensilla. Metatarsal slits are located on the middle dorsal surface, with their orientation perpendicular to the long axis of the leg. To determine the mechanical properties of the slits, we applied millinewton forces to the spider's tarsus and found that the metatarsal joint exhibits an exponential force-angle relationship, allowing spiders to detect a wider range of deflection. This indicates that the resonant frequency of this joint is a function of joint angle.

By combining these measurements, we aim to infer how spiders modulate their leg vibration sensitivity to effectively detect prey. Importantly, this study will improve our understanding of sensorimotor integration of substrate vibration sensation.

Session: Participant Symposium 6

Category: Motor systems, sensorimotor integration, and behavior

Sensory integration of visual and mechanosensory feedback in the hawkmoths, Daphnis nerii

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Flying insects must balance the demands of speed and agility with the precision of their movements to swiftly and accurately respond to environmental stimuli. Achieving this balance requires them to integrate sensory information from various modalities. Of particular importance are visual inputs from their compound eyes and mechanosensory inputs from their antennae, which are crucial for maintaining flight stability. This challenge is particularly pronounced in insects like hawkmoths, which navigate under low light conditions. Prior studies on diverse hawkmoths and other insects have highlighted the critical role of antennal mechanosensory feedback in flight control, akin to the function of halteres in flies. How is such multisensory integration achieved? We addressed this question by conducting recordings from descending neurons in the cervical connective nerve in the Oleander hawkmoths, Daphnis nerii. The moths were provided with visual stimuli comprised of moving spots of light and mechanical stimuli to their their antennae. While these stimuli were presented singly or concurrently, we recorded intracellularly from axons of descending interneurons to determine if they respond to one or both stimuli. In addition to a number of exclusively visual or mechanosensory descending neurons, we also identified several neurons that multiplex the visual and mechanosensory signals such that a single neuron encodes both visual stimuli from the compound eyes, and mechanosensory stimuli from the antennal Johnston's organs. Additional experiments at the level of behavior in intact moths reveals that integration of visual and antennal mechanosensory feedback plays a key role in gaze stabilization in flying hawkmoths. Together, these experiments underscore the importance of multisensory integration during flight in hawkmoths.

Session: Participant Symposium 6

Category: Motor systems, sensorimotor integration, and behavior

Adaptation of a Sensorimotor Internal Model to Environmental Context in Freely Swimming Electric Fish

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Internal models predicting the sensory outcomes of motor actions are crucial for sensory, motor, and cognitive functions. However, the relationship between motor actions and sensory input is intricate, varying on multiple timescales depending on both the environmental context and the animal's state within it. The neural mechanisms responsible for generating and adapting sensorimotor predictions under such challenging real-world conditions remain poorly understood. Utilizing underwater neural recordings, quantitative analysis of unconstrained behavior, and computational modeling, we investigate the function of an internal model at the initial stage of active electrosensory processing in mormyrid fish. Firstly, we demonstrate that electrosensory lobe neurons can learn and store multiple sensorimotor predictions simultaneously, specific to different states within a given environment. Subsequently, we reveal that this predictive flexibility necessitates the integration of motor-related signals with rapid electrosensory feedback. Finally, by intermittently modifying the electrical properties of the tank wall, we examine the adaptation dynamics of the internal model to changes in environmental context. These findings offer mechanistic insights into how cerebellum-like neural circuits establish and adapt internal models capable of generating complex sensorimotor predictions in dynamic environments.

Session: Participant Symposium 6

Category: Auditory system and acoustic signaling

Sound localization behavior in the Barking Gecko (Ptenopus garrulus)

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Lizards have coupled, highly directional ears, but sound localization behavior has not been observed in this clade. We therefore examined orientation in male barking geckos. Their calls serve both to attract females, and in male-male competition by enabling males to maintain exclusive space surrounding male burrows. Thus, there should be strong selection on males to orient towards the calls of conspecifics.

We measured auditory brainstem responses to sound, laser vibrometry, anatomy and orientation in barking geckos at the Kuruman River Reserve, South Africa. ABRs were measured in a sound-insulated box, and directional responses to sound were recorded using a portable laser vibrometer. Interaural transmission was computed using local sound stimulation and laser vibrometry. Sound localization behavior was recorded on video under infrared illumination. The camera was directed at the burrow mouths of calling geckos to record responses to gecko calls played sequentially from eight Bluetooth speakers at 30˚ intervals around the burrow. Orientation responses were quantified from the video recordings.

Barking geckos are very sensitive to sound in a frequency range up to 10 kHz with peak sensitivity at 3 kHz. The ear is strongly directional from 2 to 4 kHz (the peak frequencies in their calls), where interaural transmission gain is close to 0 dB, showing strong interaural coupling. Behavioral analyses showed that geckos oriented to call playbacks with an accuracy of about 8°. The accuracy was highest from sounds from frontal angles and closer to 20° for the most lateral sound sources.

The directionality of the barking gecko ear is similar to that of other lizards, but their robust sound communication behavior allows us to relate this directionality directly to behavior. This first demonstration of sound localization in lizards should support further studies of directional behavior and inform our understanding of neural processing and modeling of directional sensitivity.

Session: Participant Symposium 6

Category: Auditory system and acoustic signaling

Barn owls do have ageless ears

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In mammals, the sensory hair cells, neurones, and endolymph-generating tissue of the inner ear are all vulnerable to degenerative processes that collectively lead to a typical progression of age-related hearing loss that can be further accelerated by acoustic and ototoxic insults. In contrast, birds are renowned for their ability to regenerate sensory hair cells and re-innervate them, leading to an impressive functional recovery after such insults. The same regenerative processes have been suggested to be at work during normal ageing and are believed to underlie the remarkable preservation of behavioural threshold sensitivity shown for old starlings and barn owls – a phenomenon that has been dubbed "ageless ears". However, it remains unknown whether the auditory periphery is truly "ageless" or whether in behavioural tests the central auditory system might potentially be compensating for, and thus masking, peripheral deficits.

Here we contrast two important, functional cochlear metrics between young-adult (up to 4.5 years of age) and old barn owls (9 – 12y): Compound activity of the auditory nerve (CAP), measured at the inner-ear round window, and endocochlear potential, measured in scala media. For 5 young-adult and 6 old owls, CAP thresholds were measured in 1 kHz-steps between 1 and 10 kHz. Thresholds were not significantly different between the age groups for any of the frequencies. The endocochlear potential was determined in 7 young-adult and 6 old owls. It was not significantly different between the age groups (median young-adult: +34 mV, median old: +32 mV).

These findings suggest that birds have truly ageless ears, that is, they are able to preserve cochlear function to an advanced age – even in the barn owl, a species with extremely sensitive hearing and a specialised cochlea. Ongoing analysis will quantify CAP amplitudes and count afferent neurones of the auditory nerve, both established metrics for age-related, peripheral neuropathic changes in mammals.

Session: Participant Symposium 6

Category: Auditory system and acoustic signaling

Variation in peripheral auditory function among urban and rural soundscapes

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There is growing interest in understanding how animals cope with increasing levels of sensory pollutants in human-altered environments. In animals that communicate acoustically, several studies have uncovered how vocal signals and signaling behaviors change in response to increasing levels of anthropogenic noise. Surprisingly, we have no knowledge of whether hearing capabilities also adjust. We tested the hypothesis that, among populations with different levels of natural and anthropogenic noise, the auditory system varies in ways that facilitate signal reception. We studied eight populations of Pacific treefrogs (Pseudacris regilla) exposed to different sources and levels of natural and anthropogenic noise. First, we characterized the soundscape of these populations by obtaining hourly measurements of noise levels below 1 kHz and calculating the midline-estimating statistic of rhythm (MESOR) and amplitude, which represent the daily rhythm-adjusted mean and the variability of noise levels, respectively. We then used auditory evoked potentials to measure auditory sensitivity and the efficiency of the auditory system to extract signals from noise of subjects from these eight populations. Audiograms obtained in quiet conditions reveal that frogs from populations with higher mean levels of noise (MESOR) are less sensitive to low-frequency sounds.

Session: Participant Symposium 6

Category: Auditory system and acoustic signaling

Tag-based measurement of auditory brainstem responses during echolocation in freely swimming trained and wild toothed whales

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Echolocating animals rely on their sense of hearing to extract crucial information about their environment while actively moving through complex acoustic scenes. Empirical auditory measurements from echolocating toothed whales are limited to experiments with stationary animals, which may not reflect natural biosonar behaviour. Thus, there is a significant knowledge gap in understanding auditory reception and processing during mobile echolocation. We used a suction-cup-attached biologging tag with external surface electrodes to record auditory brainstem responses (ABRs) from four freely swimming, trained bottlenose dolphins and a wild harbor porpoise during echolocation-mediated prey capture. Tag-based ABRs were extracted by triggering on both the tagged individual's outgoing clicks and on its returning echoes during target approach. Clicking dynamics during target approach led to reduced peripheral auditory sensation of outgoing clicks while the peripheral auditory sensation of returning echoes was generally constant. Individuals often heard quiet echoes at comparable sensation levels to louder outgoing clicks and showed decreased sensation of their own clicks with increasing click rate as they approached a target. These results indicate that auditory sensory inputs dynamically change during target approach across multiple species. They also suggest the presence of a previously unspecified mechanism (or mechanisms) of gain control in these species for reducing the auditory sensation of self-produced clicks. Tag-based ABRs can be used to bridge the considerable knowledge gap created by research performed under stationary experimental arrangements and natural foraging behaviour. Moreover, the method is widely applicable to other comparably sized odontocete species, including more exotic wild species, providing unprecedented insight into toothed whale echolocation.

Session: Participant Symposium 6

Category: Electrosensory system

Hot Fish: Synchronization of behavioral rhythms by temperature in weakly elect

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Photoperiod acts as the main zeitgeber of physiological and behavioral daily rhythms, yet its unreliability in certain habitats has prompted animals to utilize other environmental cycles as alternatives. In poikilothermic animals, like the weakly electric fish Gymnotus omarorum, temperature emerges as a likely candidate. In murky, vegetation-laden waters where photoperiod cues are unreliable, G. omarorum exhibits robust nocturnal activity patterns, including heightened exploratory behavior and an increase in the rate of their electric organ discharge (EODr). These changes in EODr are a behavioral display that is modulated in response to the perceptual and social needs of the animal. To explore the role of temperature as a synchronizer, we devised a semi-natural experimental setup mimicking their habitat while enabling high-throughput positional data and EODr value acquisition. By using this set-up we were also able to eliminate photoperiodic information while maintaining environmental temperature cycles, balancing the complexity of natural environments with controlled laboratory conditions. G. omarorum displayed a daily rhythm of locomotor activity and EODr increase even when the light/dark cycle is suppressed. This showcases that periodic light cues are not necessary for these animals to maintain their behavioral rhythms. Lastly, rhythms maintained constant phase relationships with natural temperature cycles, indicating temperature as the primary circadian synchronizer in both lighting conditions, which suggests that, in this species, temperature acts as the main synchronizer of circadian rhythms. Our study illuminates the adaptive strategies of species within habitats characterized by extreme lighting conditions, and underscores the significance of understanding behavior expression under natural conditions.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Hydrostatic Pressure Modulated Cells in the Goldfish Telencephalon

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Navigation is a high order cognitive ability that is crucial for the survival of fish and almost all animals. Therefore, fish navigation is an excellent model system for understanding the development of cognitive abilities across all vertebrate classes. This study explored how depth is represented in the brains of fish, the largest group of vertebrates. Fish navigate in an aquatic environment where the density of water is about 800 times greater than air. Hence, water pressure gives fish the opportunity for direct sensing of position along the depth axis. This makes fish navigation fundamentally different from terrestrial animals. Previous study has found that goldfish encode position using boundary vector cells, measuring distance and direction from salient features in the environment. A subset of these neurons had firing rates that gradually increased or decreased with swimming depth, suggesting a gradual monotonic encoding of the fish's position in the depth dimension. Here, we investigated the contribution of hydrostatic pressure in these cells for position encoding in goldfish. We show results from two experimental assays- the first freely goldfish swimming in an invariant visual scene while varying the water level leading to changes in hydrostatic pressure. In the second experiment, we used an adjustable pressure chamber where other sensory cues remain constant. In both experiments, the neuronal activity was modulated by hydrostatic pressure variations. Using a hydrostatic cue for position encoding such as shown here provides a new perspective of place encoding in the brains of vertebrates, which might be used for fish to navigation in the underwater world.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

In-flight pinna orientation sacrifices interaural intensity difference acuity for sonar range and directionality in aerial hawking echolocating bats

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Acoustic localization is important across the animal kingdom and essential for echolocators. High frequency emission along with small head size and measurements of highly directional receiving beams are the foundation for the long-standing assumption that most bats use interaural intensity differences (IIDs) for acoustic localization in azimuth. However, bats possess flexible and movable ears and because pinna position dictates receiving beam orientation, it is crucial to consider their in-flight orientation. Utilizing a novel wind tunnel, combined with high-speed stereo video, a multi-microphone array, and head-related transfer function (HRTF) measurements, we reveal that aerial hawking vespertilionid bats (Myotis nattereri, M. daubentonii and Pipistrellus pygmaeus) focus their ears forward and directly on target during prey approach. The pinna orientation results in a substantial overlap of both receiving beams, and thus very sparse IIDs, that are inadequate to account for the reported localization accuracy of bats. However, by aligning the emission and the receiving beams, bats achieve a sharp and steep combined sonar beam which amplifies the central acoustic axis aimed at prey and strongly attenuates off-axis echoes. Our findings challenge the prevailing view that bats rely on IIDs for horizontal localization in flight. We suggest that free-flying bats optimize signal-to-noise ratio by focusing their receivers onto the acoustic axis trading IIDs for sensitivity and directionality when listening for weak echoes. The narrow acoustic field of view and on-prey focus inherently provide spatial information and combined with additional monaural and binaural cues such as interaural time differences (ITDs) could facilitate localization in the absence of large IIDs. The highly directional combined beam also provides a very simplified acoustic scene and may contribute significantly to how bats navigate and forage at high speed in complex environments so efficiently.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Comparative Connectomics of the Insect Central Complex

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The insect central complex (CX) is a brain region responsible for context dependent action selection, in particular in relation to visually guided spatial orientation. Recently, the CX connectome of the fruit fly Drosophila melanogaster has revealed extraordinarily tight structure-function links in this brain region, in which the implemented neural computations of many circuits are directly defined by the morphological projection patterns and connectivity of the involved neurons. This makes the CX a prime target for comparative analysis of neural circuit function. While the CX is fundamentally important, the demands on its circuits differ between species - depending on motor abilities, sensory environments, and behavioral strategies. To illuminate how evolution has modified these circuits to enable the enormous diversity of insect ecologies, but without disrupting the region's core functions, we have started to generate CX connectomes across the insect phylogeny, supplemented by selected crustacean species. We aim at, firstly, defining the core elements of the CX circuits to determine their likely ancestral state, and secondly, at identifying evolutionary hotspots that allow novel functions to emerge from the CX circuits. We have so far obtained EM image volumes for six species of bees and ants, a cockroach, a praying mantis, two species of dung beetles, and a locust. We combine projectomes of the entire CX with embedded local connectomes to extract key connectivity principles. We found that all core cellular components are conserved across all analyzed insects, but that even highly conserved circuits contain hotspots of high circuit diversity that could serve as access points for evolutionary change. Additionally, we have identified entire sets of neurons, in particular in the youngest parts of the CX lineages, that exist in some, but not other species, suggesting that species specific behavioral abilities could emerge from these unique elements.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Eye capping suggests eyes drive independent motor control functions during visual navigation in ants

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View based homing is a general navigation strategy in which the current view is compared to remembered views to generate a travel direction. It is often assumed that visual panoramas are stored and matched as entire views. However, modelling work has shown that the accuracy of homing can be improved if wide-field segments of the panorama are used to generate independent estimates of the correct heading. This suggests that for an ant with near panoramic vision from two eyes, using the inputs from each eye independently to derive heading directions could improve visual homing. To test this, we used an eye-capping protocol with Cataglyphis velox ants. Ants were caught on their return trip and transported to an arena in either familiar or unfamiliar locations. Prior to testing, ants were either left-eye capped, right-eye capped or subject to a sham treatment. Our null hypothesis is that the visual panoramas used by ants to navigate are stored and matched as entire views. In this case, eye-capping would have no effect on the ants' paths as the best match would involve ants adopting an orientation where the uncapped eye had the same view, and thus orientation, as experienced in training. However, we observed that eye-capping led to asymmetric paths with ant's turns being erratic when turning away from the side of the eye-cap. While this asymmetry could be a consequence of perturbing the visual homing mechanism, it could also be a result of a general change in movement control. We therefore also tested eye-capped ants released at a visually unfamiliar ground (i.e. visual homing was not a viable strategy), finding that the turning asymmetry was reduced. Together our results suggest that during visual homing the visual input to each eye may be used independently, perhaps controlling the extent of contra-lateral turns.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Hippocampal encoding of object distance in echolocating bats

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The location of environmental objects contributes to scene representation, which is incorporated into the brain's cognitive map. A posited neural instantiation of the cognitive map is the hippocampal formation, containing place cells that encode an animal's location in space. Given the importance of scene representation to spatially guided behaviors, we examined if hippocampal neurons also encode the location of objects. We investigated this question by recording hippocampal activity in echolocating bats that tracked objects moving along the distance axis. Echolocating bats emit high-frequency sounds and process the time delay between calls and echoes to estimate object distance. It is well established that bats adjust the duration and rate of sonar calls with respect to object distance, and this robust acoustic behavior yields a metric of object tracking. We recorded from CA1 of the echolocating big brown bat, Eptesicus fuscus, as it performed a sonar target tracking task in the dark. Bats were trained to perch on a platform and track a target that moved towards them. The bat's echolocation calls were recorded with an array of microphones, and its head direction was measured using a high-speed motion tracking system. Multichannel neural recordings were taken from the hippocampal CA1 of the behaving bat, synchronized with audio and video data. Spikes were sorted offline, and response areas of 154 neurons were quantified. Our data show that a population of hippocampal CA1 neurons encode the call-echo time delay (distance) to an object. These representations exist in both allocentric and egocentric coordinates. Population analyses reveal accurate decoding of object distance only with a handful of neurons. During trials when the bat ceased echolocating, the distance code degraded, suggesting that object representations depend on the bat's active sonar tracking of the object. Our results thus offer new insights into the hippocampal representation of object distance.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Neural mechanisms for a stable head direction estimate in dynamic, naturalistic visual environments

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Tracking head direction is essential for flexible, goal-directed navigation and doing so accurately over extended periods of time requires the use of sensory cues like the sun. Under natural settings, deriving a consistent sense of direction from external cues can be challenging, as cues may temporarily disappear, for example, when clouds hide the sun. Furthermore, when moving through the environment, terrestrial landmarks traverse the field of view, creating conflicts for a head direction estimate. In such dynamic environments, how can a brain extract a consistent head direction estimate?

We address this question in the fly. Using two-photon calcium imaging in head-fixed Drosophila, we monitor the fly's compass-like head direction estimate while the fly explores immersive visual virtual environments. To do this, we developed a novel virtual reality system based on the Unity game engine. We show that flies generalize their frame of reference across different simulated celestial guidance cues: sun-like spots and intensity gradients. Even in environments with approachable landmarks flies can maintain a globally consistent head direction estimate, by selectively tethering neural activity to the available global celestial cues. Thus, the fly compass system is remarkably robust to cue changes and conflicts under conditions that mimic natural scenes.

How is this robustness achieved? Through connectome analysis, we identified potential circuit motifs for selecting sensory cues to generate a robust head direction estimate in complex environments. Further, using calcium imaging we characterized how visual input neurons to the fly's "compass" circuitry dynamically represent the environment and select for reliable compass cues. Finally, we used modelling to test how neural tuning, circuit motifs for cue selection and previously described plasticity in the circuitry contribute to a robust compass estimate.

Session: Participant Symposium 7

Category: Spatial orientation and navigation

Memory processes involved in dance-distance communication of honey bees

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In the context of insect navigation, recent studies have suggested that the central complex (CX) is involved in path integration, whereas the mushroom bodies (MBs) are involved in route/visual learning & memory. In the last years we performed behavioral and molecular experiments to explore learning & memory processes involved in dance-distance communication of Apis mellifera. Extensive feeder shift experiments indicated that foragers need several flights to a new feeder location to update the distance communicated in their waggle dances (Chatterjee et al 2019). During initial visits at a new feeder location, foragers reported the feeder at an intermediate distance between the old and new feeder spots. After 30-60 min of foraging, bees finally updated dance duration for the new feeder distance. We concluded that the mismatch in dance communication is likely a consequence of confirming the reliability of experiences and weighing different memories, rather than just reflexively encoding the flight distance. We then asked whether significant changes in gene expression occur in the MBs and central brain (CB) during the period of initial visits to the new feeder position. We performed a time series RNA-seq (10min, 30min, 60min, 120min) from the MB calyxes and the CB (excluding antennal & optic lobes, and subesophageal ganglion). We identified 131 differentially expressed genes (DEGs) for MBs and 192 DEGs for CB across all time points. After 30 min, we found an upregulation of neuronal signaling genes in CB (e.g., Dop1, DopR2 & Dop3, Tyr1, mAChR) as well as genes of the cAMP-PKA-CREB, IP3-Ca2+-PKC and Wnt-signaling pathways, known to be involved in learning & memory formation. After 60 min, DEGs which got upregulated in MBs included genes involved in dopamine and glutamate signaling. Such differential regulation of memory-related genes in the CB and MBs during the initial visits to a new feeder location raises new questions about the division of functions in navigation.

Session: Participant Symposium 7

Category: Other

Innate face detectors in the endbrain of young domestic chicks

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Face perception plays an important role in social interactions for many animals, including mammals and birds. Behavioural studies have shown that both human newborns and newly-hatched face-naïve domestic chicks are spontaneously attracted towards faces and face-like stimuli comprised of three dark features representing eyes and a mouth/beak. However, previous fMRI studies on young monkeys have reported that adult-like face-selective domains in the brain develop only after exposure to faces. This implies the existence of a separate, yet unknown neural mechanism underlying innate face detection. To describe the neural correlates of this innate face preference, we performed extracellular recordings in the brain of young, face-naïve domestic chicks. Our recordings revealed neurons in a higher associative brain area of young chicks that selectively respond to a face-like configuration compared to alternative configurations or isolated facial features. Moreover, the population activity of face-selective neurons accurately encoded the face-like stimulus as a distinct category. Thus, our findings demonstrate that face detectors are present in the brain of very young animals without pre-existing experience.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Localization and tuning of face cell-like neurons in a social wasp

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Polistes fuscatus wasps possess individually distinctive color patterns on their face, which they can use to visually recognize and discriminate nestmates. Behaviorally, these wasps specialize in this visual recognition ability. Here, to investigate the neural mechanisms, we presented a large set of visual stimuli including stimuli that disambiguate wasp shape from wasp color responses, while recording extracellular activity from the optic lobe to deeper circuits in the central brain. We ask (1) if there are neural responses that are selective to conspecific wasp images, (2) where they are located, and (3) what the tuning features of neurons with highly selective responses to conspecific wasp images are. We find broad selectivity to forward-facing wasp shapes (i.e., silhouettes) across the brain, including the optic lobe. We also find highly localized neural responses in the wasp protocerebrum selective to full images of forward-facing wasps, which have color patterns. We term these forward-facing wasp units, wasp cells. Wasp cells show idiosyncratic facial tuning, tending to prefer a subset of faces or specific facial features in our dataset. Collectively this population of wasp cells exhibited a specific location both within and across animals. Together, these cells show similar responses to more similar facial patterns such that at the population response level neural distance among faces is correlated with phenotypic facial distance, suggesting a population level encoding of facial identity by wasp cells. Despite having independently evolved vision let alone facial recognition, wasp cells show remarkable parallels to the face cells found in primates, suggesting that dedicated circuits with idiosyncratic feature tuning may be critical features of visual identity recognition. Further, this system now provides a key opportunity to study how these tuning features emerge through development and how social experience may shape the key axes of these tuning properties.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Spatial reversal learning in poison frogs

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Valuable resources are scattered in complex environments and the mechanism by which animals navigate to find or relocate those resources are an important topic in animal behaviour. Sex differences in spatial abilities are usually interpreted under the adaptive specialization hypothesis which propose an enhance of memory demand in individuals with highly ecological demands. During parental care, poison frogs routinely transport their tadpoles from terrestrial clutches to suitable deposition sites, and some individuals return to supply their offspring with nutritive unfertilized eggs. Here, we study differences in spatial reversal learning in two poison frog species with sex-specific parental roles to test the hypothesis that spatial cognition skill is likely to co-evolve with parental care that requires spatial tasks. We trained Dendrobates auratus (male parental care) and Oophaga pumilio (asymmetrical biparental care) to find a goal using an intramaze visual cue in a plus maze under laboratory conditions. Since males D. auratus perform most parental care duties which involves navigation in large areas to find rearing sites, I expected males to have better spatial abilities than females. In O. pumilio, females transport tadpoles and provide egg feeding while males care the eggs. As females remember the tadpole site location during provisioning, we expect better abilities in females. We found that both species learned to find a goal during training, but there was no sex difference in both species. We found no sex differences in the number of days to reach the learning criterion during training and the time spent in the correct arm during the probe trial after training. Interestingly during reversal learning, we found that O. pumilio frogs did not learn the new configuration. In D. auratus, males exhibit greater cognitive flexibility. Taken together, our results suggest that high demands on parental care duties may be associated with high flexible learning.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Wings of Change: plasticity in steering motor neurons underlies operant self-learning in Drosophila

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Motor learning is central to human existence, such as learning to speak or walk, sports moves, or rehabilitation after injury. Evidence suggests that all forms of motor learning share an evolutionarily conserved molecular plasticity pathway. Here, we present novel insights into the neural processes underlying operant self-learning, a form of motor learning in the fruit fly Drosophila. This form of plasticity may have evolved to allow flies to adapt to wing asymmetries and fly straight despite asymmetric wing kinematics.

We operantly trained wild type and transgenic Drosophila fruit flies, tethered at the torque meter, in a motor learning task that required them to initiate and maintain turning maneuvers around their vertical body axis (yaw torque). We combined this behavioral experiment with transgenic peptide expression, CRISPR/Cas9-mediated, spatio-temporally controlled gene knock-out and confocal microscopy.

We find that expression of atypical protein kinase C (aPKC) in direct wing steering motoneurons co-expressing the transcription factor FoxP is necessary for this type of motor learning and that aPKC likely acts via non-canonical pathways. We also found that it takes more than a week for CRISPR/Cas9-mediated knockout of FoxP in adult animals to impair motor learning, suggesting that adult FoxP expression is required for operant self-learning.

Our experiments suggest that, for operant self-learning, a type of motor learning in Drosophila, co-expression of atypical protein kinase C (aPKC) and the transcription factor FoxP is necessary in direct wing steering motoneurons. Some of these neurons control the wing beat amplitude when generating optomotor responses, and we have discovered modulation of optomotor behavior after operant self-learning. We also discovered that aPKC likely acts via non-canonical pathways and that FoxP expression is also required in adult flies.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Need to remember: Dolphins recall their own actions after long delays

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Human episodic memory often involves recalling one's actions in specific past situations, while prospective memory entails remembering intended future actions and executing them at the right moment. Research on memory for own actions in non-human animals remains limited, although working with trained animals opens up new possibilities for studying these memory types. The few studies investigating prospective memory in animals have focused on appropriately timed recall of a single to-be-performed action instead of variable actions. We targeted both memory systems, instructing dolphins to remember specific actions randomly assigned by trainers and later reenact them after various delays. In the first experiment the dolphins (N=6) recalled their own actions after at least 16 hours in a total of 18 trials distributed in 7 phases with increasingly time delays. In contrast, when not instructed to remember beforehand but merely asked to repeat the previous action after a set delay, they retained the memory for only 12 seconds when tested in 12 sessions with 16 trials per session (N=3). This suggests intentional encoding of actions into long-term memory, when needed in the future, which is indicative of prospective memory. The dolphins' performance also exhibits other key features of episodic memories. Their memory is declarative, as the action itself declares the memory content, and it is encoded in a single episode. Moreover, the results indicate some similar biases in dolphin and human memory systems. In a third experiment we also found that dolphins display the human-typical "enactment effect" finding it easier to recall actions they had performed themselves than to remember verbal instructions for these actions (hand signs for dolphins). This study suggests that dolphins have declarative long-term memories of their own actions, as well as prospective memories related to them.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Extending the Q-learning reinforcement learning algorithm to investigate the role of selective attention in animal movements

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The study of animal movements has benefited from the use of machine learning and artificial intelligence (AI) in recent years, encompassing for instance the development of tracking methods and of machine learning models inferring the social interactions underlying collective movements of animal groups. Another avenue of research using tools from AI constitutes a normative research programme in which agents, imbued with cognitive abilities, learn rules of movement from first principles (foraging, predation, or reproduction success). One specific contribution of this body of work is to provide a framework to test the influence of cognition on animal movements. In the context of animal movement, such framework allows to test hypotheses regarding cognitive processes and abilities such as memory, information processing or aggregation on emerging movement behaviours. Here, we present a theoretical study that investigates how selective attention affects learning and movements in animals. In most existing models of animal movements, agents pay attention to all available stimuli and move in a direction obtained after averaging over reactions to all stimuli. There is, however, empirical evidence in fish that individuals moving in groups only pay attention to a few social stimuli. We developed an extension of the Q-learning reinforcement learning algorithm in which agents learn both which stimuli matter in their environment and how to react to them with movement decisions. We introduce this new selective attention algorithm and its benefits for the study of the cognitive bases of movement in animals, with a grid-based model. Our results suggest that selective attention is particularly benefiting agents in an environment where reward (e.g. food) perception and consumption are asynchronous. We discuss the relevance of our results and future research in the light of empirical data in zebrafish (Danio rerio).

Session: Participant Symposium 8

Category: Learning, memory and cognition

Learning to Fly: the role of experience on flight behaviours in juvenile zebra finches

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Flight is the most complex form of locomotion found in the animal kingdom, as it requires considerable motor control and energy expenditure. Many birds are highly skilled and agile in flight but, despite recent technology advances allowing high-resolution tracking of birds during flight, how they acquire complex flight behaviours remains poorly understood. In particular, few studies have investigated how birds learn to cope with wind gusts and avoid collisions in high-risk scenarios.

We investigate the effect of early life experience in juvenile zebra finches on their ability to navigate sudden tail winds during a perching task. Using a motion capture system, a fan to generate controlled wind conditions, and a target perch fit with a force balance sensor, we captured landing flights in 40-day-old zebra finches which were either naive to wind or had been reared in windy conditions. We find that, after repeated attempts, all birds were able to anticipate wind and converged towards a stereotypical sideways manoeuvre to land safely on a perch while in the presence of a tailwind. However, naive birds showed a poorer performance during the perching task than birds which had prior experience coping with wind. These results provide insights into the mechanisms by which birds learn to avoid collisions and land effectively in natural environments, which suggests that rapid motor learning in the early days after fledging is likely crucial for survival in juvenile birds.

Session: Participant Symposium 8

Category: Learning, memory and cognition

Primates Persist Where Mice Explore: An Interspecies Comparison of Decision-Making

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Persisting through burdens and challenges that lack immediate rewards can eventually lead to outcomes that are more rewarding. For example, persisting in learning to play an instrument results in playing complex pieces of music. A lack of persistence is known to cause various challenges, such as difficulty in maintaining attention (Amsel, 1990), and having impulsive choices (Zermatten et al., 2005). Although persistence has been studied independently in different species, it remains unknown whether different species would mirror this persistence behaviour in an identical decision-making environment or, if instead, they would show unique behaviours due to their differences in ecological niches or the brain structure or function that evolved in those niches.

To determine if different species have different patterns of persistence, mice, monkeys and humans performed a classic explore/exploit task known as a restless multi-armed bandit. In this task, species were presented with a series of trials where they had to make choices between identical targets. Each target's reward chance changed independently over time. In consequence, all three species were presented with a conundrum: should they persist in exploiting an already rewarding option or should they explore new alternative options?

All species performed the task similarly, alternating between rapidly switching between the options, and persisting in choosing the same option repeatedly. Despite these similarities, humans and monkeys persisted in their choices for longer, compared to mice. Modelled analysis of exploration and exploitation with a hidden Markov model revealed that both humans and monkeys relied similarly more on exploitation, persisting for a longer time, whereas mice relied more on exploration. We speculate that the similarities in persistence patterns in humans and monkeys, as opposed to mice, may be linked to variations in their behavioural ecologies, neural timescales and/or cognitive self-control.

Session: Participant Symposium 8

Category: Other

Sensory processing and the socioecology of predatory ants

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Ponerine ants are renowned predators. Some species are socially basic (small colonies of monomorphic workers that forage independently), whereas others are socially complex (large colonies of polymorphic, task-specialized workers). Most ponerine ants prey on diverse invertebrates as solitary huntresses; some specialize on termites, an energetically rich clumped food source. The dietary shift from randomly distributed to clumped prey, changes the sensory demands of foraging, for example, visual navigation by solitary huntresses to chemical recruitment in termite specialists to organize group predation as well as olfactory discrimination of prey. Socioecological differences in predation are striking in the Neotropical ant genus Neoponera. We examined the size and structure of the compound eyes, antennal glomeruli, mushroom body (MB) microglomeruli (MG), and mosaic brain scaling in the sister species N. commutata, and N. apicalis. Our results indicate that N. apicalis has a larger number of ommatidia, antennal glomeruli, allometrically larger antennal and optic lobes. These sensory traits were associated with an increase in the density of MG. In N. apicalis, MG density was higher in both the MB lip and the collar, areas associated with processing olfactory and visual information, respectively. The increase in MG density in N. apicalis may be associated with higher demands for navigation, learning, and memory, as well as a higher density of glomeruli, likely involved in odor discrimination, which could be important for identifying prey. In contrast, N. commutata exhibited a larger MB which may be associated with their unique foraging strategies, and an increase in size of the antennal glomeruli and MG, which appears to be linked to their chemical communication. These findings suggest that differences in behavioral performance demands associated with socioecology are reflected in variation in eye structure, brain mosaicism, and the organization of glomeruli and MG.

The importance of time and natural light for behavior and how humans impact on this

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Our work focusses on the molecular mechanisms of moon-controlled timing systems and their interactions with the circadian clock. In many marine organisms, like the bristle worm Platynereis dumerillii, moonlight sets the phase of an endogenous monthly oscillator. Some recent questions we have been tackling are: How can different worms across a population reliably synchronize to the same moon phase? How is lunar timing influencing daily timing? Can the study of molecular timing mechanisms of marine bristle worms help to understand some of the scientifically reported, but “just weird” correlations between human physiological/behavioral rhythms and the lunar cycle? Our work on marine invertebrates also provides new perspectives on daily timing mechanisms from a habitats and species groups that differ significantly from the conventional molecular model systems confined to land. It finally also provide insight how artificial light may impact on organisms, whose temporal coordination critically depends on the right light at the right time.

Next-generation movement monitoring with deep learning and generative AI

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The past decade has brought a revolution in scientific inference with deep learning. In this talk, I will highlight how the field of computer vision has had a major impact on neuroscience and animal behavioral analysis with markerless pose estimation. Specifically, I will focus on our work developing DeepLabCut, a state-of-the-art open-source Python package for building tailored neural networks. Additionally, I will discuss ongoing efforts to build large-scale foundation models across species and how generative AI is poised to transform data analysis using natural language.

Oral Communications
Friday, 02 August 2024

Session: Plenary 8

Neuroethology of aerial predation with compound eyes

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The modular nature of compound eyes results in highly variable designs that act as 'matched filters', suited for different lifestyles. i.e. the sensory apparatus and the underlying neural system must be matched to the behavioral habits of the animal. In the case of predators, such optimization must also take into account the habits of their prey and the environment in which the engagement takes place. For aerial species, the selection pressure is even higher, as small changes in sensorimotor conversions can quickly lead to disastrous consequences. In this talk, I will review our work across different visually guided aerial predators that boast compound eyes, and highlight what we have learned about their target detection, visual tracking, prediction, decision making and prey interception.

Causes and Consequences of Sleep Disruption in Cityliving Wildlife

Author: Dr. John Lesku, La Trobe University

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Many animals live in human-modified environments. However, these outwardly expanding habitats may disrupt key behavioural and physiological processes, such as sleep. While wild spaces enjoy natural day/light cycles, with only astronomical sources of illumination, and are relatively quiet and clean; cities are quite different. Well lit, round-the-clock; loud with the sounds generated by people, transport, construction and industry. Even urban waterways are not untouched from the impacts of human activity, with trace antidepressants in city streams. Here, I explain how various forms of pollution impact sleep in terrestrial and aquatic wildlife and, where possible, identify the consequences for waking performance.

Some don't like it hot: Exploring the effects of temperature on insect pollinator sensing and behaviour

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Increasing global temperatures and, in particular, the increasing number of heatwaves, threaten important insect pollinator groups such as bees and butterflies. However, we still understand little about how temperature affects how these insects sense and process information about their environment or how this affects their behaviour and interaction with plants. We have been working to address this by investigating how exposure to elevated ambient temperatures affects physiology, sensing, cognition, foraging and pollination behaviour of bumblebees. Our results suggest that the effects of heat on these aspects have significant implications for bumblebee behaviour and pollination outcomes. This work is not only relevant for pollination studies but highlights some important knowledge gaps in our understanding of how insect sensory, neural and behavioural systems will respond to increasing temperatures.

Vulnerability of bats to anthropogenic noise

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Accumulating evidence in the past two decades has placed anthropogenic noise as global pollution that threatens various taxa of wildlife. Nevertheless, far less research and conservation attention has been given to understanding the impact of anthropogenic noise on bats. Bats are species-diverse, ecologically important, and highly mobile mammals distributing widely in all major terrestrial landscapes. Here, we used metaanalysis and a modeling approach to understand the vulnerability of bats to anthropogenic noise. The metaanalysis showed that only 60 species, representing 4.3% of extant bat species, have been tested for anthropogenic noise effects and most research occurred in the past decade. Statistical analyses revealed that the type of anthropogenic noise, foraging mode, and behavioral task were important predictors of bats' responses. Species that passively listen to prey-generated sounds during foraging are particularly vulnerable to traffic noise, suggesting that auditory masking is a primary mechanism for bats' responses to anthropogenic noise. To assess and compare the effects of anthropogenic noise on different species, we designed a sensation-based metric called the masking potential. Applying the masking potential to a global dataset of bats confirmed auditory masking as an explanation for their sensitivity to highway traffic noise. We further show that masking potential allows for estimating the impact range of noise on species with hearing sensitivity data (i.e., the audiogram). The masking potential predicted maximum impact ranges of a median of 38 m for 73 species of bats. The maximum impact ranges predicted by masking potential were supported by empirical measurements. Together, these data showed that bats are not immune to the disturbances of anthropogenic noise, despite their predominant reliance on ultrasonic vocalizations and hearing.

Session: Invited Symposium 9

Environmental noise statistics modulates sound encoding in rat auditory cortical neurons

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Cortical sound representation can be altered in response to environmental sound statistics and behavioral task engagement. Here we investigated if different environmental noise statistics can alter sound encoding in auditory cortical neurons using juvenile and adult rats. We, first, exposed Sprague-Dawley rats to spectrotemporally modulated noise at a moderate level (~60 dB SPL) from their hearing onset (P6) throughout their critical period (P45). Once these animals reached adulthood, they were trained to detect vocalizations presented in the modulated noises and compared to unexposed animals. Noise exposure enhanced the behavioral performance of detecting rat vocalizations in background noise. Next, we investigated neural responses in the primary auditory cortex (A1) and ventral auditory field (VAF) to evaluate spectrotemporal response properties and the ability of signal-in-noise processing. Noise-exposed animals improved cortical signal encoding compared to unexposed control animals. Moreover, receptive field properties showed specific shifts away from the noise statistics in the noise-exposed animals. Finally, we conducted noise exposure in adult rats from P60 to ~P112 to investigate if adult animals express plasticity comparable to that observed during the critical period. We found, indeed, similar effects of enhanced signalin-noise processing and shifted modulation representations in the cortical neurons although it was a more moderate degree. These findings support that environmental sound statistics can affect hearing ability throughout the lifetime with potential implications for understanding neural mechanisms of adaptation to the noisy environment. It is important for animals to be able to hear meaningful foreground sounds in different types of backgrounds. Therefore, the ability to adaptively shift cortical receptive fields away from the prevalent noise parameters to enhance signal-in-noise processing provides a useful tool for survival.

Session: Invited Symposium 9

What's the trouble with noisy neighbours? – Impacts of chronic noise on songbird physiology and behaviour

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Human activities such as transport and industry generate huge amounts of noise, and noise levels continue to increase and spread globally such that very few places on Earth are free from anthropogenic noise. The severity of this noise pollution as a health concern for humans is clear, with the WHO warning that noise is the second biggest cause of environmental health problems in the world, after air pollution. Noise pollution also impact exposed wildlife, although the extent to which noise reduces fitness is not well understood. Noise can negatively impact fitness through disruption of acoustic communication but can also have non-auditory effects on the physiology and behaviour of exposed individuals. In the lab we experimentally exposed breeding birds to chronic playback of traffic noise at realistic levels for urban dwelling birds, and investigated a suite of physiological and behavioural traits, including sleep, telomere loss, metabolic hormone levels, reproductive behaviour, offspring mortality, physical development, vocal behaviour and learning. We find that noise has pervasive, multi-faceted and cross-generational effects on the health and development of birds, from significant delays in learning and growth to reduced immune function and poor sleep quality. We also find that even brief bouts of noise exposure during critical life-history stages, such as breeding or early development, can have long-term consequences that are transmitted to subsequent generations. While the long-term fitness implications of these studies for wild populations is not fully understood, growing evidence suggests that chronic noise pollution can lead to long-lasting population-wide shifts in genes and behaviour, leading to cascading effects at the community level.

Session: Invited Symposium 10

Category: Other

Mental time travel in the rat

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Mental time travel involves reexperiencing the past and imagining the future. Theoretical perspectives focus on autonoetic consciousness and chronesthesia. Because phenomenology is not assessed in nonhumans, comparative studies take a behavioral perspective, asking what an animal capable of mental time travel can do via its behavior. The central hypothesis of animal models of episodic memory is that, at the moment of a memory assessment, the animal remembers back in time to a specific earlier event. Tests of this hypothesis suggest that rats are a suitable model of episodic memory. Here, I outline the perspective that rats are capable of mental time travel. A prediction of mental time travel is that the animal searches its representations in episodic memory in sequential order to find information. I review research that demonstrates that rats are capable of searching representations in episodic memory to find specific event memories, rules out the use of memory-trace strength and working memory, shows that this ability is hippocampal dependent, and shows that rats replay incidentally encoded episodic memories. I argue that searching the contents of memory is a form of mental time travel in nonhumans that is relatively tractable because it focuses on the contents of memory.

Session: Invited Symposium 10

Category: Learning, memory and cognition

Do dogs “remember”?

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Episodic memory is considered as the conscious recollection of personally experienced events, is thought to involve mental time travel and is typically assessed with language-based reports. The existence of episodic memory in non-human species has been studied with different methodologies, reflecting diverse theoretical approaches. Since it is challenging to assess consciousness and mental time travel, studies in non-human species focus on the behavioural aspects of episodic memory. We investigated this form of memory in dogs in the framework of definitions proposing episodic memory as recalling after incidental encoding, which can be assessed if the recall test is unexpected. We capitalised on dogs’ imitative skills to devise tests that would rely on this capacity to “ask them questions” about past events. We tested whether dogs could rely on episodic memory when recalling their owners’ actions. Dogs trained to imitate with the Do as I Do method were tested on imitation of their owners’ actions in unexpected recall tests. Later we investigated whether dogs remember their own spontaneous actions relying on episodic-like memory. Dogs were first trained to repeat a small set of actions upon request. Then we tested their ability to repeat other actions produced by themselves, including actions performed spontaneously in everyday situations – when a test was not expected. Dogs remembered and imitated their owners’ and their own actions, also in the unexpected tests, showing evidence of episodic-like memory. The combined evidence of representing own actions and using episodic-like memory shows a richer representation of a key feature of the self than previously attributed to dogs. These methodological approaches allowed unprecedented investigations of memory of complex, context-rich events. Taken together, the results of studies on dogs and other species may question the necessity of including consciousness and mental time travel in the identification of episodic memory.

Session: Invited Symposium 10

Category: Other

Mental time travels: insights from cephalopod molluscs

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Symposium Unexpected Mental Time Travels

Mental time travels: insights from cephalopod molluscs

Christelle Jozet-Alves

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Mental time travel is considered as the ability to mentally travel backward or forward in time: remembering past events (episodic memory), as well as imagining possible future ones. Since the complex cognitive abilities of modern cephalopod molluscs (e.g. cuttlefish and octopuses) have arisen independently of the vertebrate lineage, they appeared over the past years as highly promising species to study mental time travels from evolutionary, mechanistic, functional and comparative perspectives. cuttlefish. We showed that cuttlefish keep track of what they have eaten, where and how long ago (unique what-when-where events), in order to match their foraging behaviour with the rate of replenishing of different food items. Cuttlefish are also able to determine the origin of these memories: they can retrieve the perceptive features belonging to the memory of a previous event (namely whether they have seen/smelled an item) when unexpectedly asked. Cuttlefish not only adapt their foraging behaviour to different replenishing rates, but also to the availability of their preferred food in the proximate future. Indeed, they reduce their crab consumption during daytime when their preferred prey is predictably available the following night (availability on alternate nights). While cuttlefish are commonly described in literature as opportunistic predators, this range of experiments shows that they can adopt day-to-day flexible foraging behaviours including selective, opportunistic and future-dependent strategies.

Session: Invited Symposium 10

Category: Learning, memory and cognition

Parrots remember accidentally encoded own actions

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Episodic memory, i.e., the recall of personally experienced episodes is difficult to assess nonverbally. We present a new method to study it in non-human animals using an unexpected question paradigm inspired by Fugazza et al. (2020). We worked with six behaviorally trained blue-throated macaws (Ara glaucogularis) that reliably displayed three different trained actions upon specific hand commands given by an experimenter. In two separate previous studies the macaws had been trained to reliably respond to two further gestural commands, namely “repeat” and “copy”. Receiving the “repeat” command they reenacted their last action which they had performed on command shortly before. When seeing the “copy” command, they copied the action a conspecific placed next to them had displayed. Hence, in one context the subjects anticipated to repeat their own previous action and in the other to copy a conspecific’s action, but the contexts had never been intermixed previously. We carried out unexpected recall tests by embedding a “repeat” request into three different “copy” sessions in which the macaws imitated the actions of a conspecific. We scored successful unexpected repeats and analyzed gaze duration during expected and unexpected trials. Two out of six parrots remembered their own last action in 3/3 and 2/3 unexpected repeat trials respectively. Two individuals had just one trial correct and two individuals failed completely performing random actions or just copying the other bird. Gaze durations were longer in unexpected compared to expected repeat trials. The immediate successful performance of two individuals suggests that macaws have the capacity to recall specific own past actions encoded implicitely. The other birds’ failure may reflect the difficulty of the task which is further evidenced by the observed violation of expectation effect. Parrots should be included into the study of mental time travel and the presented method is suited for comparative studies.

Session: Invited Symposium 11

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Evolution and development of insect vibration receptor organs

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Substrate vibrations are ubiquitous stimuli in the habitat of animals, and numerous insect species produce communication signals based on such vibrations transmitted in abiotic substrates like soil or leaves and branches of plants. Insects detect substrate vibrations by different types of sensilla distributed over their body. Chordotonal organs consisting of scolopidial sensilla are commonly the most sensitive receptor organs for substrate vibrations. They are in most cases located in the insects' legs, which transfer vibrations from the substrate.

The subgenual organ in the tibia is an important vibration receptor organ present in most insects. In orthopteroid insects like crickets, cockroaches, or stick insects, are additional chordotonal organs present next to the subgenual organ, forming the subgenual organ complex. This increases the complexity of the sensory apparatus by including several vibrosensory organs, increases in the numbers of sensilla, or different orientations of sensory neurons. The additional organs may have different functional morphologies and physiological properties.

In Hemimetabola including orthopteroid insects, the chordotonal sensilla usually differentiate during embryonic development. The subgenual organ complex is present in newly hatched insects, when vibrational stimuli from the environment (e.g., predators) become behaviourally relevant. The functional morphology in early larval instar stages resembles that of adult insects, indicating the responsiveness to vibrational stimuli. This is confirmed by electrophysiological recordings in different stages of larval instars. Notably, changes in the organisation of sensilla in the chordotonal organs can occur. This may relate to a more efficient detection of vibrational stimuli, and could indicate morphological changes to gain physiological responses during postembryonic development.

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Session: Invited Symposium 11

Category: Other

Multimodal communication in crickets and katydids

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Male crickets and katydids are well-known for their acoustic signals, which are used to attract and court females for mating. Vibrational signalling is common in these species as well. The relationships between these signaling modalities, however, are not as well-studied. Here I will present results of several studies looking at the interplay between acoustic and vibrational signaling in crickets and katydids.

Broadcast signaling to attract a mate is usually accomplished through acoustic signaling in these animals, but in one subfamily of katydids (Pseudophyllinae), males will also spontaneously produce vibrational signals. Females that detect male vibrational signals respond similarly as when they detect acoustic signals, by producing their own vibrational signals and/or approaching the male. In a phylogenetically controlled study, we found a negative relationship between the amount of acoustic and vibrational signaling by males across species, suggesting a trade-off between the use of two signal types with overlapping functions.

Most crickets in the family Gryllidae show categorical perception based on sound frequency, moving towards sounds 12 kHz (typical of bat echolocation calls). The neural bases for these behaviours are well-known. In one clade (tribe Lebinthini), however, males produce high-frequency acoustic signals, females reply to male calls with a vibrational signal, and males use the vibrational signals to locate the females. We tested two hypotheses for the origin of this acoustic-vibrational duet, that the female vibrational reply originated from a startle response to high frequency sounds or that it evolved from high frequency calls and vibrational signalling present during courtship. Although not mutually exclusive, behavioural and neural evidence suggest that the origin for this unique communication system is more likely to be startle than courtship behaviour.

Session: Invited Symposium 11

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

What sets the communication frequency in arthropod communication?

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Many invertebrates use sound and vibration to communicate, typically producing relatively narrowband calls to signal to prospective mates. Acoustic communities appear to maintain efficient signalling networks by using acoustic niche separation. In this theory, senders and receivers have matched filters for sound production and reception and maintain a communication channel. Additionally, species that use the same acoustic space appear to avoid each other's frequency channel. Indeed, niche separation predicts that animals will through exclusion end up using a wide range of frequencies and acoustically active species have call frequencies that span two orders of magnitude.

In contrast, we see a significantly narrower range of frequencies in vibrationally active species like spiders. Here we suggest that this is because spiders sense vibrations using slit sensillae, sensors that depend on leg joint function. Leg joints are multi-functional and are also critical to locomotion. The biomechanics of these joints need to be tuned to enable them to support the spider's mass but will also set perceivable vibration frequency range. Thus, multifunctionality narrows the frequency space available to vibrational communicators. To test this hypothesis, we studied 11 spider species and measured the stiffness of two joints on two legs. Using mixed effects models, we examined the scaling relationships between joint stiffness and spider mass. We found that heavier spiders had stiffer joints, and the relationship between mass and stiffness was nearly allometric. These data when incorporated into biomechanical models suggest that spiders of different body masses will nonetheless be mechanically stimulated by very similar vibration frequency ranges. Thus, our data suggest that using the whole body as a sensor prevents spiders from niche partitioning the vibrational signal space.

Session: Invited Symposium 11

Category: Auditory system and acoustic signaling

Balanced network activity for the processing of structured acoustics signals in bushcrickets

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Acoustic insects use sound and vibration signals to find mating partners. Different strategies of acoustic communication have evolved in the family of bushcrickets/katydids (Tettigoniidae). We study how complex signals, such as the conspecific song, are processed in the ears and along the ascending auditory pathway. In the bushcricket Mecopoda elongata, a structured broad-band song consists of 10–14 pulses with increasing amplitude. In this study, we use biomechanical measurements to investigate the processing of conspecific acoustic signals in the ear of M. elongata, the crista acustica. Our results indicate that the encoding of communication signals is modulated by ear mechanics.

To understand the spiking pattern induced by conspecific song, we systematically characterized the neuronal processing along the auditory pathway using a combination of different electrophysiological methods (intracellular single cell recordings and extracellular hook- and multielectrode recordings). Our multielectrode-recordings in the prothoracic ganglion provide evidence that the neuronal network in the prothoracic ganglion is balanced between excitatory and inhibitory inputs to encode the temporal structure of the chirp. Comparative analysis of spike rates in response to the entire chirp and the single pulses of the chirp, point to a strong inhibition in the network that leads to decreased spike rates in the chirp. This inhibition helps to preserve the temporal structure of the song. Further, we show that the temporal dynamics of synaptic activity, measured by local field potentials, are characterized by a long-lasting increase in the amplitudes that correlate with the identified spiking pattern (spike rates and first-time spiking). Both processes, namely spiking and local field potentials, play a crucial role for the temporal processing of the calling song during acoustic communication in M. elongata.

Session: Invited Symposium 12

Category: Metabolism, biological rhythms and homeostasis

The influence of the social environment on the brain circadian transcriptome of two social bees differing in the level of social complexity

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Social insects provide an attractive model system for studying natural socially-regulated plasticity in circadian rhythms because task performance and the social environment influence the expression of circadian rhythms. We tested the influence of brood care behavior and colony environment on the whole brain transcriptome in two highly social bee species showing different levels of social complexity. The Western honey bee Apis mellifera is highly social with perennial colonies containing tens of thousands of individuals, whereas colonies of the bumble bee Bombus terrestris are annual and contain up to a few hundred worker bees. We combined behavioral observations and time-series brain RNA sequencing to study the influence of the colony environment and brood tending on circadian gene expression. We sampled bees experiencing four different social environments: Foragers (colony, no brood care), nurses (colony + brood care), individually isolated ("solitary") worker bees (no colony or brood care), and solitary bees with brood (brood care, no colony environment). To identify circadian-regulated genes and molecular processes, we performed circadian analyses, multivariate analysis, WGCNA, and enrichment analyses. In both species, the canonical "clock genes" were cycling as found in previous studies, validating our experimental design and RNA sequencing procedures. As expected, in both species we found significantly more circadian-regulated genes in behaviorally rhythmic foragers and the lowest number of cycling transcripts in nurses that care for the brood around the clock. Surprisingly however, the solitary with brood group had the second highest number of circadian-regulated genes, which suggests that factors in the colony other than the brood have strong influence on circadianly regulated processes in the brain. We will discuss genes and pathways that are circadian-regulated by the colony environment and brood care.

Session: Invited Symposium 12

Category: Metabolism, biological rhythms and homeostasis

Sexual Dimorphism in the Drosophila Circadian System

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The circadian clock is essential for regulating a wide range of physiological and behavioral processes, including sex-specific or sexually dimorphic behaviors. In Drosophila as in other species, most of the research on how the timekeeping system in the brain controls timing of behavioral outputs has been conducted in males, or sex was not included as a biological variable. The main circadian pacemaker neurons in Drosophila release the neuropeptide Pigment Dispersing Factor (PDF), which functions as a key synchronizing factor in the network with complex effects on other clock neurons. Lack of Pdf results in most flies displaying arrhythmicity in activity-rest cycles under constant conditions. However, our results show that female circadian rhythms are substantially less affected by mutations in both Pdf and PdfR. To determine if M cell outputs that are independent of Pdf are also sexually dimorphic, we manipulated activity and gene expression of their main postsynaptic partners based on connectomics data. Finally, we tested the influence of the Pdf+ cells ("Morning" cells) over the circadian network and show that speeding up the molecular clock specifically in these cells leads to sexually dimorphic phenotypes both under light dark cycles and under constant conditions, with a more pronounced effect on male rhythmic behavior. Our results suggest that the M cells are less dominant over the female than the male circadian network and that circadian timekeeping is more distributed across the clock neuron network in females.

Session: Invited Symposium 12

Category: Metabolism, biological rhythms and homeostasis

Aquatic Night Owls? Behavioral Rhythms of African Electric Fish

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Electric fish are generally considered nocturnal animals because they can use their non-visual active electrosense for navigation, foraging, and communication. However, activity patterns have only been described for a few species, and usually under controlled light regimes. Many natural habitats of electric fish are characterized by poor visibility and low light levels throughout the diel cycle. Other abiotic environmental fluctuations (e.g., in dissolved oxygen and temperature) and biotic factors (e.g., social interaction, predation pressure) likely play important roles as well for the activity patterns of electric fish. This raises the question whether nocturnality persists in complex natural settings. We built autonomous recording devices and deployed them in different habitats of four species of African electric fish in Uganda to monitor their electric organ discharges throughout the diel cycle. We recorded light intensity, temperature, dissolved oxygen content, pH, and conductivity in these habitats to identify factors affecting natural activity patterns. Overall, the study species showed the highest swimming activity and were recorded most often in groups at night. However, fish that occur commonly in social groups retained elevated activity levels in constantly dark habitats even during the day. Our data give insight into natural patterns of activity and social interaction and suggest that light intensity and social lifestyle likely shape activity patterns that deviate from the often-assumed nocturnality of electric fish.

Session: Invited Symposium 12

Category: Other

Temperature and social context modulate daily rhythms of behavior in the wild

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Natural behavior occurs concurrently with a variety of stimuli among which daily environmental cycles play a pivotal role in synchronizing endogenous circadian rhythms with the environment. The allocation of activity and rest in specific moments of the day enhances survival rates while optimizing energy expenditure. Social interactions rely on this temporal organization since individuals need to coincide with conespecifics in order to engage in social behavior. Gymnotus omarorum, is a nocturnal pulse type South American gymnotiform. Their characteristic electric discharge (EOD) sereve sensory and communication purposes and results from the spontaneous activity of a medullary pacemaker modulated by environmental, physiological and social information. Nocturnal arousal in this species is marked by an increase in the rate of emission of EODs (electric behavior, EOD-BR) alongside with increase exploratory activity and social interactions. Fish inhabit freshwater ponds and live in a mostly dark environment due to the high turbidity of the water and the thick floating vegetation which blocks daylight. Despite the irregularity of daylight exposure EOD-BR recorded in the wild exhibits a robust daily cycle tightly associated to natural thermoperiod. Animals occupy different positions under the vegetation patch mostly sheltering among a thick mass of roots, defending individual territories all year round. Despite territoriality, social context exerts a synchronizing role in the daily cycle of EOD-rate which highlights the advantage of activity synchronization among conspecifics. Results from in vitro, and behavioral experiments, both in laboratory and nature are put together with the aim of understanding the joint action of social and environmental modulation on the daily rhythm of electric behavior. Temperature synchronization and social modulation of daily rhythms emerge as key factors in the timing of this natural rhythmic behavior.

Session: Plenary 9

Collective puzzle solving by ants and humans

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Collective intelligence is a captivating aspect of group dynamics. But are groups really more “intelligent” than individuals? Since groups and individuals tend to interact with the world at different scales – this question often makes little meaning. An exception comes from ants and humans, two of the rare species that can transport loads not only alone but also as part of a group. While hauling large loads relies on joint muscle power, navigating them through complex environments can benefit from collective cognitive abilities In this talk I will describe how individuals and groups of either ants or people tackle scaled versions of an identical geometric load maneuvering puzzle. By examining how problem-solving skills vary with group size in each species, I will draw comparisons between them.

Session: Invited Symposium 13

Category: Motor systems, sensorimotor integration, and behavior

Electrophysiology and motor performance in octopus

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Studying motor behaviors can reveal fundamental principles of the brain and mind. This presentation focuses on our work towards developing the hardware and computational tools that will allow scientist to measure movement of an individual in a dynamic environment. The octopus has many features that makes it advantageous for pursuing a holistic understanding of motor control and behavior and mapping central and peripheral neural circuits. We will show novel technologies to record electrophysiology signals from octopus's nervous system and the potential of new computer vision platforms to measure octopus movement with high resolution. Using these approaches, we can start modeling octopus arm movement with high precision. These methods could improve brain-machine interface neuroprostheses and help develop new assistive technologies that will allow compensation and augmentation of specific circuits.

Session: Invited Symposium 13

Category: Motor systems, sensorimotor integration, and behavior

The neural basis of cuttlefish camouflage

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(This is part of the Cephalopod Neuroethology symposium)

Cuttlefish are coleoid cephalopods that dynamically change the color, pattern and texture of their skin to camouflage with their surroundings. Camouflage is achieved by expanding and contracting pigment-filled saccules in the skin called chromatophores, through the action of motor neurons that project from the brain. Thus, the patterning of the skin is a physical manifestation of neural activity in the brain. We are using this system to understand how the physical properties of the visual world are represented by patterns of neural activity in the brain, and how this representation is transformed into an approximation of the physical world on the skin. We have performed a series of experiments to develop the dwarf cuttlefish, Sepia bandensis, as a model to investigate the neural basis of camouflage. We have described the stages of embryonic development, sequenced the genome and neural transcriptome, completed a 3D brain atlas, and developed a visually-evoked camouflage behavioral paradigm. Furthermore, we are generating transgenic cuttlefish that express genetically-encoded calcium indicators and light-activated channels, permitting the live imaging and manipulation of neural activity. These technologies should permit us to simultaneously record neural activity and measure behavior to uncover how visual information is deconstructed in the brain, and then reconstructed into an image of the physical world on the skin.

Session: Invited Symposium 13

Category: Evolution and development

Death and the octopus: neuroendocrine control of reproduction and lifespan in Octopus bimaculoides

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In octopuses, reproduction and lifespan are tightly controlled by the optic glands, a functional analog of the vertebrate pituitary gland. Optic gland signaling is responsible for a series of post-mating behavioral changes that invariably leads to death. Though this phenomenon has been referenced by cephalopod biologists since at least the beginning of the 20th century, optic gland function has only recently begun to come into focus. Here, I highlight advances in our understanding of the neuroendocrinology of post-reproductive death and dying in the octopus.

Session: Participant Symposium 9

Category: Auditory system and acoustic signaling

A Bat's Tale of Auditory Processing and Social Communication

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Bats are auditory specialists, processing acoustic signals to guide their behaviors, including prey tracking, navigation, and communication. Most bat species are very social and emit a wide array of communication calls, including food-claiming calls, aggressive calls, and appeasement calls. There is strong evidence that in humans and other animals, social context plays a role in the processing of acoustic signals. Yet, the circuits and mechanisms that govern this process are still not fully understood. Bats emerge as outstanding mammalian models to explore the neural mechanisms underlying acoustic communication processing. Here, I provide an overview of our work related to how bats analyze, and process signals used for communication and I will introduce the approaches we are taking in my lab to explore this avenue of research. In particular, focusing on our recent work on the social dynamics of the bat Carollia perspicillata. This species of bat has fission-fusion social dynamics, where animals may change roosts on a nightly basis, nevertheless, our results show that these bats will prefer to roost with a known conspecific than with a novel individual. Currently, we are evaluating the neural responses along the auditory pathway to the different types of emitted calls to further understand how these signals drive behavior.

Session: Participant Symposium 9

Category: Auditory system and acoustic signaling

Unbiased whole-brain calcium imaging reveals parallel and sequential auditory processing and specific tuning to vocalizations in a transparent and vocalizing teleost, Danionella cerebrum

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Sounds are highly informative about environmental events near and far and constitute an integral element of social behavior. Yet, our understanding of how vertebrate brains detect and sequentially transform auditory cues into neuronal representations facilitating behavioral decisions is still very limited for largely technical reasons. Here, we developed and applied technology for unbiased, volumetric brain-wide recordings of neuronal activity under finely controlled acoustic stimulation. The males of the small and transparent teleost species Danionella cerebrum generate pulsed social sounds that are extremely rich in temporal structure and occur in sequences of short bursts or in continuous bursts with durations of seconds up to minutes. Imaging auditory responses in males and females we identified the major elements of the their auditory pathway from hindbrain to pallium at cellular resolution. Surprisingly, the auditory system segregates the processing of tones and vocalizations sharply into separate channels early in the ascending auditory pathway. Within the vocalization-selective pathway, the response profiles of auditory brain areas successively narrow down to a few specific vocalization types, with the thalamic and preoptic regions displaying high specificities for distinct vocalizations types. Interestingly, we find that auditory response profiles up to the auditory thalamus were remarkably similar across males and females.

Session: Participant Symposium 9

Category: Auditory system and acoustic signaling

Neural and behavioral evolution in an eavesdropper with a rapidly evolving host

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The diversification of animal communication systems is driven by the interacting effects of signalers, intended receivers, unintended receivers, and the environment. Yet, the critical role of unintended receivers like eavesdropping enemies in signal evolution has received little attention. Furthermore, the contemporary evolution of novel animal signals is rare, making it difficult to directly observe the role of unintended receivers in this process. Female parasitoid flies of the genus Ormia are obligate parasitoids and rely exclusively on acoustic cues to locate singing male orthopterans. In Hawaii, selection imposed by Ormia ochracea has led to recent and rapid phenotypic diversification of new songs in their local cricket host, Teleogryllus oceanicus. We use a complementary set of lab and field experiments to compare the neural and behavioral responses of Hawaiian flies to those of an ancestral fly population to understand the role of receiver psychology in the evolution of novel host songs. We demonstrate that introduced Hawaiian flies have evolved differences in neural auditory tuning and behavior that likely facilitate the detection of novel host songs. Hawaiian flies prefer novel song variants with certain characteristics, enabling us to make predictions about how selection imposed by the fly may shape song features in the future. Our findings provide a rare example of rapid evolution in the sensory tuning of an eavesdropper to correspond with a rapidly evolving host.

Session: Participant Symposium 9

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Evolution of temperature preference behavior in Drosophila

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How preference for a given temperature range evolves during the colonization of new environments is not known. Here, we show that at least two distinct neurobiological mechanisms drive the evolution of temperature preference in flies of the genus Drosophila. Fly species from mild climates (such as D. melanogaster and D. persimilis) avoid heat, and we show that this can be fully explained by differences in the activation threshold of peripheral hot receptor neurons. In contrast, desert-dwelling D. mojavensis are instead attracted to heat. We demonstrate that this is due to a valence switch, from aversive to attractive, in how the brain processes input from the peripheral receptors. Although insects are ubiquitous, few species inhabit thermal extremes. Our findings illustrate how adaptation to desert life in Drosophila involved a remarkable rewiring of the thermosensory system.

Session: Participant Symposium 9

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

The precise sense of time of harbor seals

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Time constitutes one of the fundamental parameters of every existence, influencing behaviors ranging from short time intervals to annual cycles. Time perception has already been studied in many terrestrial animals. However, the sense of time of marine mammals, such as harbor seals, has been scarcely studied to date, even though it could play a role in a variety of behaviors in a variety of contexts, such as during foraging, orientation and navigation. The first experiments on time perception in pinnipeds revealed that harbor seals have a precise sense of time when discriminating optically marked time intervals in the second and millisecond range. Since there is not always a stimulus available that carries the time information in the seals' marine habitat, we wanted to determine whether the seals possess intrinsic timing abilities. We thus set out to investigate, whether harbor seals can learn a temporal discrimination task in the absence of visual and, more generally, any sensory stimulation during the time interval by using empty intervals. Different to filled intervals, that were used in the first seal timing studies, empty intervals are only marked by a very short optic stimulus at the beginning and the end of the interval, but not during the interval. Our experiment showed that harbor seals are able to time empty time intervals in the second range. They can discriminate the empty intervals comparable to filled intervals with a difference threshold of 0.85 sec for a standard time interval of 3 sec. This suggests that harbor seals can utilize intrinsic time information, when external time cues are not available, a condition, which harbor seals might often experience in their environment. Consequently, we expect harbor seals to have access to temporal information, while navigating through the ocean consistently.

Session: Participant Symposium 9

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Bees learn local cues differently than distal panorama cues in a spatial memory task

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Bees are excellent navigators. As central place foragers, they must return to their hive from different locations and results from radar experiments suggested that bees navigate by using a cognitive map. However, which visual features are stored in the insect brain to facilitate navigation remains unclear. Behavioral results show that the skyline panorama guides insects and models predict that visual panoramic snapshots are stored in the insect brain. To return to the goal location, insects seek to match the current retinal image with a visual snapshot stored in the goal's vicinity. These results imply that insects do not differ between local and distal cues but rather all landmarks are part of a panoramic snapshot. To test whether insects indeed use local cues in a same way as distal cues, we designed a spatial memory task. In a circular LED arena, bees had to learn the location of a rewarded feeder. In a dual choice paradigm, the bees had to choose between two visually identical feeders. Only one of the feeders contained sucrose solution. Our results show that the bees learned the location of the rewarded feeder by using panoramic cues presented at the arena's wall and proximal, three-dimensional landmarks placed on the arena's floor. Surprisingly, bees differently responded to novel local cues than to novel distal cues added to the panorama. Moreover, when setting local and distal cues in conflict, the honeybees' decision primarily depended on the local cues, a cue hierarchy that could be switched, when only distal cues reliably signalled the goal location. Our results are consistent with findings from the hippocampal formation of vertebrates and suggest that there exist two memory traces in the bee brain, one for learning local cues and the other meant for distal cues. With tetrode recordings from the brain of freely walking bees, we currently aim to find the neural correlates of landmark and and panorama processing.

Session: Participant Symposium 10

Category: Methods and education

Creating 3D Brain Atlases of Poison Frogs and Swordtail Fish

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Comparative neuroscience relies heavily on well established brain atlases and modeling of brain structures, which currently only exist for classically studied organisms, such as mice, zebrafish, and humans. However, expanding these toolkits for emerging models can provide a spatial reference tool for improved neuroscience research by enhancing our understanding of neuroanatomy, brain function, and gene expression by allowing visualization of the whole brain on a 3D scale. Finally, brain atlases across the vertebrate lineage – and within closely related species – enable comparisons of brain structure and function across evolutionary timescales. 3D brain atlases do not currently exist in swordtail fish or poison frogs, two emerging animal models in neuroethology. To remedy this, we used immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) to visualize brains of Mimic Poison Frog (Ranitomeya imitator) tadpoles and El Abra swordtails (Xiphophorus nigrensis). We have labeled landmark neuroanatomical markers and imaged our brains with lightsheet microscopy to render a species representative template. This will allow for strengthened future findings in the neuroscience studies of these animals, and more broadly, establish pipelines for other neuroethologists working with emerging organisms and developing brain atlases in their own labs.

Session: Participant Symposium 10

Category: Methods and education

YORU: Animal behavior recognition system with object detection algorithm

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Understanding the neural basis of social interaction behavior is an important topic in neurobiology. The well-regulated spatiotemporal manipulation of neural activity, as animals exhibit specific behaviors of interest, serves as a powerful approach to study the causal relationship between neural circuits and behaviors. This approach requires real-time detection of each individual's behavior. Recently, markerless body parts tracking analysis has been proposed for use in closed-loop systems to provide real-time feedback based on the animal's behavior. However, the application of such systems to social behaviors, in which multiple individuals interact dynamically, remains challenging. Therefore, novel methods are required to analyze social behaviors in order to provide real-time feedback in response to the behaviors.

Here, we propose the use of an object detection algorithm (YOLOv5) based on a deep neural network, to identify social behaviors consisting of multiple individuals. Our idea is to use this algorithm to classify the behavior of individuals based on their appearance as a "behavior object". First, we validated the effectiveness of the algorithm on social behaviors ranging from vertebrates to insects. Second, we integrated the object detection algorithm with a control system for operating optogenetic devices and packaged it into a graphical user interface called "YORU". Finally, as a practical demonstration of YORU's capabilities, we utilized the real-time feedback system to selectively disrupt the mating interaction of fruit flies by individual- and behavior-specific optogenetic interventions. YORU allowed us to deliver photostimulation only to specific individuals during the behavior, even when multiple individuals were present in a single chamber. The system was successful in significantly reducing the copulation acceptance rate of female flies, demonstrating the utility of the YORU system in providing real-time feedback in response to social behavior.

Session: Participant Symposium 10

Category: Vision and photoreception

The visuomotor transformations underlying defensive behaviors and hunting

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Identifying visual stimuli as potential predators or prey and generating an appropriate response is a fundamental task of the nervous system. Specific types of visual stimuli can evoke hunting and various defensive behaviors; small moving objects can trigger hunting, a rapidly looming disk mimics an approaching predator and triggers escape, while moving disks of constant size represent a potential but not imminent threat, and trigger freezing. However, it remains largely unknown how these stimuli are transformed into the proper behavior.

Here, we show that larval zebrafish can respond to sweeping visual objects of constant size by freezing. The stimulus causes immobility, and a reduction in heart rate, or bradycardia, another signature of freezing. We found that, as in other species, dark moving disks are the optimal stimuli for freezing. We then mapped the neurons involved in this behavior, as well as hunting and escape, using volumetric 2p imaging to record from two-thirds of the larval brain while presenting behaviorally relevant stimuli and recording behaviors. We found that all three stimuli activated sensory neurons in the tectum, and identified separate classes of behavior-correlated sensorimotor neurons within the tectum. We found that the tectum transforms relatively broad sensory responses into highly selective sensorimotor responses, which could allow flexible and context-dependent behavior. We also identified sensorimotor neurons in downstream areas thought to be involved in fear in other animal models, suggesting that these circuits are deeply conserved. The three sensorimotor populations were almost completely segregated, suggesting that the decision of which behavior to execute is established at the level of the sensorimotor transformation in the tectum.

Session: Participant Symposium 10

Category: Vision and photoreception

Seeing the vibrant colours underwater – unravelling the spectral processing and colour constancy of mantis shrimps' colour vision

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Stomatopods, commonly known as mantis shrimps, possess one of the most complex visual systems in the animal kingdom. Their compound eye is divided into two hemispheres by six rows of enlarged ommatidia, termed the midband. The first four rows (Rows 1-4) of the midband are responsible for their unique colour vision, featuring up to 12 photoreceptor classes with narrow-band sensitivities from deep UV to far red. While most visual systems require only 2-4 spectral sensitivities, the processing mechanism and function behind stomatopods' colour vision remain unclear.

Based on the neuroarchitecture, it was initially hypothesized that Rows 1-4 may function as a multiple spectral opponency system, where signals are compared within each row, enabling fine spectral discrimination (1-3 nm) and colour constancy. However, previous behavioural study revealed low spectral discrimination abilities (15-20 nm) in stomatopods. This rather surprising finding suggests that their colour vision may also employ an unconventional processing mechanism called the binning system, which bins information into separate channels for rapid colour recognition.

While recent anatomical and behavioural studies have suggested the coexistence of both mechanisms in their vision, no evidence beyond anatomical and modelling levels has been found to support the spectral opponency system and colour constancy in stomatopods' vision. To address this gap, we investigated further the spectral processing and colour constancy of the stomatopod Haptosquilla trispinosa.

Through electrophysiological, behavioural, and immunohistological approaches, our results suggest spectral opponency at the first optic lobe of stomatopods and that Rows 1-4 can operate as a multi-spectral opponency system. Furthermore, we revealed that this unique system allows stomatopods to exhibit exceptional colour constancy, surpassing that of most animals, including humans.

Session: Participant Symposium 10

Category: Vision and photoreception

High-resolution vision in a pelagic polychaete – Why have alciopid worms evolved enormous eyes?

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High-resolution vision, the ability to segment and identify specific objects in the environment, has only conclusively evolved in three of the over thirty-four animal phyla: vertebrates, arthropods and mollusks (specifically cephalopods). However, there is now evidence that some polychaete worms are capable of high-resolution vision. While most polychaetes have diminutive eyes with limited visual capability, alciopids (Phyllodocidae) have a pair of bulbous eyes ten-times larger than their heads. These worms live an enigmatic life in the open ocean and can be difficult to collect in good condition and high numbers.

Recently, we have identified a field site at Ponza, IT, where we can reliably collect healthy individuals from three species by hand. This has allowed us to examine the optical and physiological basis of their visual system. We found that the alciopids eyes have the spatial and temporal acuity for high-resolution vision, adding a new thread to the evolutionary story of vision and emphasizing the rapidity at which new eyes can evolve [1]. However, it remains unknown why alciopids have gone this route. Many of their phyllodocid cousins also live pelagic lifestyles but have much lower-performing eyes. Hypotheses for the elaboration of alciopid vision include as a means of detecting transparent prey, avoiding predators, or finding each other to mate. Their high nocturnal activity level could also suggest a function in detecting bioluminescence. Here we present new molecular, physiological and behavioral evidence that resolves some of the mysteries of alciopid visual behaviors, and offers tantalizing clues about why they have evolved such spectacular eyes.

1. Bok MJ, A Macali & A Garm (2024) High-resolution vision in pelagic polychaetes. Current Biology 34, R269–R270. 10.1016/j.cub.2024.02.055.

Session: Participant Symposium 10

Category: Vision and photoreception

Jumping spider vision uses a conserved set of three opsins with diverse protein localization patterns across species

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The principal eyes of jumping spiders (Salticidae) integrate a dual-lens system, a tiered retinal matrix with multiple photoreceptor classes and muscular control of retinal movements to form high resolution images, extract color information, and dynamically evaluate visual scenes. Despite this dynamic visual system and the independent lineages evolving elaborate signaling colors, little work has been done to characterize the opsin diversity and retinal localizations among eye pairs and across species. Transcriptomic data across 80 salticid species representing 22 tribes revealed a set of three conserved visual opsins (Rh1 - green sensitive, Rh2 - blue sensitive, and Rh3 - ultraviolet sensitive), with evidence for opsin gene duplications in only 8 species. Immunohistochemistry (IHC) studies of these three opsins across six species in five tribes found that while localization in the primary eyes of all species followed previously published patterns with RH1 in two proximal tiers and RH3 in two distal tiers, in at least one species RH2 was co-localized across all four tiers suggesting an expanded set of color sensitivities. Even more surprising, the patterns of each opsin in the secondary eyes was distinct in each eye type and species. In particular, the lateral eyes were dominated by RH1 opsins, while the posterior median retinas were dominated by opsins forming short-wavelength sensitive visual pigments (e.g. RH2 and/or RH3). There was also variation among species in the distribution of opsins in retinal photoreceptors among secondary eye types, as well as labelled extraocular locations, particularly for the putatively blue-sensitive visual pigment formed from RH2. Our findings suggest secondary eyes have the potential for color vision, with observed differences between species likely associated with different ecologies and visual tasks.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration, and behavior

Daily vocal exercise is necessary for peak performance singing in a songbird

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Vocal signals, including human speech and birdsong, are produced by highly complicated, precisely coordinated body movements and peak vocal performance is fitness determining in resource competition and mate choice. While the acquisition and maintenance of motor skills generally requires practice to develop and maintain both motor circuitry and muscle performance, it is unknown whether vocal, like limb muscles, exhibit exercise-induced plasticity. We showed that juvenile and adult songbirds require intense daily vocal exercise to first gain and subsequently maintain peak vocal muscle performance. Preventing singing rapidly altered muscle and vocal performance and females preferred song of vocally exercised males. Vocal output thus contains information on recent exercise status, suggesting it is an honest indicator of past exercise investment in songbirds, and possibly in all vocalizing vertebrates. To estimate the timespan that is reflected in song, we followed vocal recovery after singing prevention in adult male zebra finches. We quantified changes in fundamental frequency, entropy and source level over the course of 3 weeks. Our data allows us to estimate for how long and how far back in time vocal output of a male could reveal his past singing exercise.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration, and behavior

A sensorimotor understanding of spider orb-web construction

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Many innate behaviors are the result of several coordinated sensorimotor programs to produce higher-order behaviors. Knowing the underlying cognitive states that encode how these programs are coordinated is often difficult since we simply can't ask the animal their objective. However, extended phenotypes such as architecture provide us with a window into the mind because the structure itself is a physical record of behavioral intent. A particularly elegant and easily quantifiable structure is the spider orb-web. We have developed a novel assay enabling high-resolution behavioral quantification of web-building by the hackled orb-weaver Uloborus diversus. With a brain the size of a fly's, the spider U. diversus offers a tractable organism for the study of complex behaviors. Using machine vision algorithms for limb tracking and web annotation, we have developed an atlas of stereotyped movements and sensorimotor transformations that ultimately produce the web. The rules for how these movements are coordinated change during different phases of web construction, and we find that we can predict web-building stages based on these rules alone. Thus, the physical structures of the web explicitly represent distinct phases of behavior. To uncover how this sophisticated algorithm is encoded in the brain, we have assembled a genome, a brain atlas, and have developed biological assays such as EASI-FISH, immediate early genes (IEGs), RNAi, drug delivery, and live calcium imaging, to identify which neurons and genes are critical to encoding web-building behavior.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration and behavior

Sleep disruption improves behavioral performance in zebrafish larvae

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Sleep deprivation is known to drastically affect cognitive function including decision-making and attention across many different species. Here, we leveraged the small size and conserved brain structure of larval zebrafish to investigate the consequences of sleep disruption in the context of two well-described behaviors, a visual and an olfactory-based decision-making task. We find that in both paradigms, sleep disruption leads to an improvement in performance. Specifically, we show that sleep disruption increases reaction time and improves performance in a visual motion discrimination task, an effect that we attribute to longer integration periods in disturbed animals. We also find that sleep disruption leads to increased odor sensitivity, which we show is likely mediated by cortisol. Building on our previous research, our work allows us to predict specific circuit changes underlying these effects. Our findings set the groundwork for further investigation of the underlying circuit changes in the brain that occur as a result of sleep disturbance across different species.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration, and behavior

The surprising pulsating soft coral: where neurobiology meets the reef

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The unique, hypnotic pulsation of xeniid corals has fascinated scientists since Lamarck. However, the neural mechanism of repetitive pulsation in these animals, likely to be controlled by a pacemaker, has never been investigated. As such, Xenia umbellata, a fast-growing Red Sea octocoral, was developed as a laboratory model to explore the mechanism of pulsation in xeniid octocorals. Our recent study has revealed that this organism possesses rapid regenerative abilities. During oral disc regeneration, the pulsation behavior develops in stages, indicating stepwise regeneration of the pacemaker. By utilizing this feature of behavior development throughout regeneration, we set out to reveal aspects of how X. umbellata performs this unique behavior. Using transcriptomic and immunohistochemical analysis, we have explored the molecular mechanism of pulsation, as well as the structural development of the neuromuscular system. Using differential expression analyses, candidate ion channels, neurotransmitters, and transcription factors were identified that correlate with the development of pulsation behavior, and the likely location of the pacemaking unit was identified. Our research should open the door to a deeper understanding of pacemaker functioning in these intriguing corals.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration, and behavior

Octopus visual perception of motion

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Cephalopods, a diverse class of marine mollusks, are among the most intelligent invertebrates. Their highly centralized nervous system, often defined as a proper 'brain', underpins a compelling richness of behaviors and learning abilities. With many cephalopod species being fast moving active predators, that can collect information from several sensory modalities, they present a fascinating model for studying the evolutionary principles underlying neural circuit design and behavioral specialization. The study of many aspects of motor behavior in cephalopods has increased in the last 15 years. The octopus is an especially interesting model with a soft body and eight highly flexible, muscular hydrostat arms. However, despite the wealth of knowledge regarding their soft bodies, arm usage and neural control, a fundamental component of motor cognition remains unexplored: the visual perception of motion. Recognition of movement and motion prediction are crucial for survival and adaptability in motile animals, and serve as an ontogenetic and phylogenetic foundation for social cognition. In this study, we used a simultaneous discrimination learning task to test motion perception in Octopus vulgaris. We created a novel psychophysics, behavioral testing arena, in which two targets, black circles on a grey background, could be presented simultaneously on a single video screen, at the far end of the tank. This video presentation allowed us to show identical targets which differ only in velocity. Over several training stages, octopuses learned to approach the target moving at the correct velocity to retrieve a food reward. These results indicate that Octopus vulgaris can specifically discriminate the velocity component of motion and use it in a learning task. This study opens the door to further investigation into motion and action perception in octopuses, establishing octopuses as comparative models for visuo-motor circuits and neural computation of motion in invertebrates.

Session: Participant Symposium 11

Category: Motor systems, sensorimotor integration, and behavior

Residual vocal capabilities and diverse recovery patterns after brain injury revealed by social context

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Complex learned motor sequences, such as speaking or bird song, involve combining simple movements into sequences. Such sequences are controlled by complex patterns of neural activity distributed across many brain regions. What residual motor skills remain after brain injury and how such motor skills recover, remain poorly understood. Here, we addressed this question by recording and analyzing the songs of adult male zebra finches after partial lesions of HVC, a premotor nucleus essential for normal song production. As reported in other studies, partial lesions of HVC (n=10 birds with ~30-80% lesion) disrupted the acoustic structure and sequence of songs produced when the bird was alone. Surprisingly, songs directed towards a female were more similar to pre-lesion songs in both acoustic structure and song sequence. This difference in song properties across the two social contexts (“alone” song vs. female-directed song) was much larger than normal differences present in adult birds. Over a 1-2 week period post lesion, songs stabilized and became similar in both contexts. However recovery of the original pre-lesion sequence was not uniform; some birds only sang a part of their pre-lesion sequence immediately after lesion and slowly added back syllables at the end (n=3 birds) while other birds started out with their entire pre-lesion sequence and gradually lost syllables (n=6 birds). The effects of lesions could also be replicated by partial inactivation of HVC with muscimol (n=4 birds) and could be rescued by simultaneous inactivation of LMAN (n=2 birds), the output nucleus of a songbird basal ganglia-thalamo-cortical pathway important for song plasticity, ruling out the involvement of circuit re-organization in the recovery process. Overall, our results highlight the potential of social context as a tool to understand current motor capabilities and recovery trajectories after brain injury.

Poster Session I

Poster Session 1 | Poster Wall 1 | Label: PS1.001

Category: Auditory system and acoustic signaling

How the auditory brainstem of bats detects regularity deviations in a naturalistic stimulation paradigm

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Bats, like all mammals, rely on the identification of regulations and deviations in their acoustic environment. This phenomenon, called deviance detection, has been studied intensively in the past and keeps gaining attention in the field of electrophysiology. Over time, an impressive complexity of deviance detection could be shown, in both animal and human studies. However, complex forms of auditory deviance detection were so far only demonstrated for high-level brain structures. Here, we used a naturalistic acoustic stimulation protocol to demonstrate that complex deviance detection already happens in the lowest stations of the auditory pathway, the brainstem. These potentially feedback mediated effects could contribute significantly to the saving of resources very early in the processing of acoustic sounds.

Poster Session 1 | Poster Wall 2 | Label: PS1.002

Category: Auditory system and acoustic signaling

Prey detection strategy in echolocating bats – Doppler shift compensation as a S/N improving strategy –

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Echolocating bats emit ultrasonic pulses and listen to the echoes to perceive their surroundings. During the flight, the echo frequency increases due to the Doppler effect caused by the relative velocity between the bat and the echo target, although some bat species decrease the pulse frequency to cancel this effect and keep the echoes at a constant frequency. This Doppler shift compensation (DSC) behavior is thought to be a strategy for receiving echoes in their auditory highly sensitive frequency. However, as echoes from prey are not compensated for, the true significance of this behavior is not yet clear, and here in this study, we reexamined the meaning of this behavior. First, we testify the feature of echoes targeted by horseshoe bats in this behavior with playback experiments of artificially frequency-shifted echoes to the bats. The results confirmed that the bats target the "highest frequency" echoes, not the "highest sound pressure" echoes, for DSC. Next, we mounted an on-board microphone on the bats and measured the echoes while they were attacking their prey moths to clarify the actual echo frequency bats receive during flight by performing DSC. The acoustic data showed that performing DSC on the highest frequency echoes results in a "quiet frequency zone" with no echoes at all above the compensated frequency. Furthermore, acoustic glints reflected by the fluttering moth's wing were observed in this quiet zone. Finally, to confirm the importance of this quiet zone, we playback band noise when bats trying to find prey moths. As a result, when this quiet zone is being disturbed by noise, bats were much less likely to find and attack the moths than when the lower frequency is being disturbed. These findings suggest that DSC is not a behavior to listen to echoes at a constant frequency, but rather a strategy to prevent cluttered echoes from appearing in the frequency band for moth detection and to detect the glints from prey with a good signal-to-noise ratio.

Poster Session 1 | Poster Wall 3 | Label: PS1.003

Category: Auditory system and acoustic signaling

Combination of 3D photogrammetry and the boundary element method as a noninvasive method of modelling head related transfer functions in bats

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Echolocating bats navigate and forage by emitting high frequency sound pulses to localize and identify objects in their surroundings from the returning echoes. As such, bats rely heavily on accurate sound localization. Animals localize sounds through binaural and monoaural cues. One key monoaural cue for bats is difference in the spectrum of sounds reaching the ear which stems from sounds entering the ear with different admittance depending on the frequency and direction in relation to the pinna. These are the spatio-spectral monoaural cues which can be described through the head related transfer function (HRTF). Traditionally, the HRTF of bats are measured experimentally by inserting a microphone into the ear of a dead animal and recording the sounds from speakers placed around the bat. More recent approaches use µCT scanners to create 3D models which enable numerical modelling of the HRTF. We propose the use of 3D photogrammetry as an inexpensive alternative to µCT scanning and combine it with the boundary element method to simulate the HRTF of bats. The accuracy of the 3D model from photogrammetry is evaluated by comparing with a µCT scan, where we achieved accuracy of ±50µm for the test case of a 3D printed bat head. Our results show good overall correspondence between photogrammetry based HRTF when comparing with both experimentally measured HRTFs and µCT based HRTFs. There is strong indication that photogrammetry is a viable alternative to µCT scanning for this purpose. Photogrammetry also has the potential of capturing the 3D model of live animals, opening to new possibilities regarding natural position of the pinna, and performing previously impossible large-scale studies.

Poster Session 1 | Poster Wall 4 | Label: PS1.004

Category: Auditory system and acoustic signaling

Neural substrate for echo delay and phase perception in FM bats

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Psychophysical experiments demonstrate that echolocating big brown bats can detect changes in the phase of FM echoes as a component of delay, and they have enhanced detection for echoes that alternate in phase compared to echoes that are fixed in phase. To accommodate ultrasonic phase in delay percepts, echo delay acuity is of the order of 1 μs. The bat's ability to perceive echo phase shifts as corresponding changes in delay means that the effective latency variability of on- or off-responses at some stage of auditory processing must be no greater than 10-15 μs, not >100-300 μs as estimated from studies in other mammals. Otherwise, pooling of delay information across neurons would obscure the presence of phase shifts. We recorded local field potentials (LFPs) from the inferior colliculus (IC) to tone bursts varying in phase. Responses to frequencies of 10-16 kHz proved sensitive to changes in stimulus phase. This phase sensitivity was manifested in the off-response, not the on-response. Phase sensitivity in the off-response was greatest for short envelope fall times, suggesting that the phase of spectral splatter is represented in the off-responses. We also recorded LFPs in the cochlear nucleus (CN) and in the IC to echo cascades – FM sweeps followed at certain time delays by a series of echoes. In both brain areas, we observed latency variability to the FM sweep and to the first echo in the cascade in the range of 10-200 μs, considerably more precise than that observed in single neuron responses. This sharp latency coding is still less precise than expected from the bat's behavior. The question remains as to what intervening computations in other brain regions reinstate the short latency variability needed to understand the bat's percept. Work supported by ONR grant N00014-171-2736

Poster Session 1 | Poster Wall 5 | Label: PS1.005

Category: Auditory system and acoustic signaling

In-air and underwater hearing of turtles and tortoises from four families

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Since the chelonids (turtles and tortoises) evolved from diapsid ancestors, they have undergone many evolutionary changes. In the course of evolution they developed (independently) a tympanic middle ear, but either retained or secondarily reverted to an ancestral-type amniote auditory papilla. Sensitivity of hearing has only been studied in very few of the 171 species, and most of what we know about chelonian hearing is based on one species – the red-eared slider Trachemys scripta. Recent studies have demonstrated many instances of sound production in turtles and tortoises and supposed uses of these sounds in communication, urging a more comparative study of chelonid hearing.

In this project we measured auditory brainstem responses from seven different species of chelonians and four different families. Two of the species were aquatic (Chelodina longicollis, Cyclemys sulcata), three were semiaquatic (Trachemys scripta, Cuora flavomarginata, Cuora amboinensis) and two were terrestrial tortoises (Centrochelys sulcata, Chelonoidis carbonaria). We recorded responses from anesthetized animals stimulated with airborne and underwater sound. We also measured eardrum vibrations of these species using laser vibrometry. All species had very similar audiograms in air, with best frequencies at 2-400 Hz and lowest thresholds around 40 dB SPL. In water, the best frequencies also were from 200-400 Hz with lowest thresholds at 90 dB re 1 uPa. Eardrum vibrations at the most sensitive frequencies ranged from -20 to -10 dB re 1 mm/s/Pa.

We show a surprisingly uniform sensitivity of hearing, generally independent of lifestyle (aquatic/terrestrial). The sensitivity to frequencies above 1 kHz is low, and some of the supposed communication sounds are relatively high-frequency and therefore unlikely to be heard. Since it is very easy to produce sounds, especially under water, caution is needed when assuming sound communication based on sound recordings 'in the presence of animals'.

Poster Session 1 | Poster Wall 6 | Label: PS1.006

Category: Auditory system and acoustic signaling

Sound perception differs between sexes in a wild songbird during a vocal cooperative behavior – duet singing

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Many organisms coordinate rhythmic motor actions with those of a partner to generate cooperative social behavior, such as duet singing. While it has recently been shown that vocal premotor activity is synchronized between the brains of vocally cooperating individuals, the sensory mechanisms of such behavior remain unknown. What kind of acoustic information are duetting individuals listening to in order to produce a well-coordinated duet song, their own vocalizations, those of the partner, or both? To tackle this question, we telemetrically recorded extracellular, multiunit activity in the auditory cortex of wild, male and female White-browed sparrow weavers (Plocepasser mahali) while the birds produced antiphonal duet songs in their natural habitat. In parallel to the neural activity, we recorded the individual vocal activity of both duet partners with wireless microphones attached to each bird. We found a remarkable difference in auditory processing between males and females during singing: While auditory units in male birds only responded to the bird's own vocal emissions, auditory activity in female birds correlated with both, own song syllables and syllables produced by the male partner. This led us to conclude that female but not male birds listen to the partner's vocalizations during duetting.
To test this hypothesis, we performed playback experiments with syntax-manipulated duet songs. While both sexes were able to duet with the playback of correct versions of their partner's part of the duet, only females were able to adjust their own vocal output to changes in syllable order in manipulated versions of the male duet part. This difference in auditory perception may represent the basis for the formation of leader-follower roles in vocal cooperative behavior.

Poster Session 1 | Poster Wall 7 | Label: PS1.007

Category: Auditory system and acoustic signaling

Bat auditory brainstem response recovery cycles reflect species differences in echolocation call rate

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Echolocating bats rely on precise auditory temporal processing to detect echoes generated by calls that may be emitted at rates reaching 150-200 Hz. High call rates can introduce forward masking perceptual effects that interfere with echo detection; however, bats may have evolved specializations to prevent repetition suppression of auditory responses and facilitate detection of sounds separated by brief intervals. We assessed post-stimulus recovery of the auditory brainstem response (ABR) in two species that differ in the temporal characteristics of their echolocation behaviors: Eptesicus fuscus, which uses high call rates to capture prey, and Carollia perspicillata, which uses lower call rates to avoid obstacles and forage for fruit. We observed significant species differences in the effects of forward masking in which E. fuscus maintained comparable ABR wave 1 amplitudes when stimulated at intervals.

Poster Session 1 | Poster Wall 8 | Label: PS1.008

Category: Auditory system and acoustic signaling

A multiomic approach to the molecular basis of swarming in the African malaria mosquito

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Swarming is an incredibly important behaviour in the life history of the African malaria mosquito, An. gambiae. As novel insecticides are increasingly needed to fight malaria, the temporal and spatial regularity of swarms makes them a desirable target for mosquito control. Despite this, swarming is understudied and the molecular control of many components of this complex behaviour are unknown. To begin unravelling the neurological control of swarming, we utilised a mutiomics approach to investigate the male An. gambiae brain; combining transcriptomics and metabolomics to identify transcripts and metabolites that are differentially expressed over the course of a day and increase around swarming time, with a special interest in G coupled protein receptors. We have identified several neurotransmitter receptors that fit these criteria. Of those receptors, the dopamine and ecdysone receptor, AgDopEcR, has shown the most promise of having a significant role in the control of swarming behaviour. We are now studying this receptor through RNA interference and its effects on a variety of behaviours involved in swarming such as flight tone, activity level, and mating success. Additionally, we are investigating the role of dopamine and ecdysone in hearing, which is an important component in male mating finding.

Poster Session 1 | Poster Wall 9 | Label: PS1.009

Category: Auditory system and acoustic signaling

Modulation of syllable period-selective phonotaxis in female cricket Acheta domesticus

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Selective phonotaxis exhibited by female cricket Acheta domesticus and Gryllus bimaculatus has been documented in multiple studies. While some females are finely tuned and attracted to calling songs with syllable periods matching those of the natural calls of the conspecific male, other females respond phonotactically to calls with a wider range of syllable periods. Nanoinjections of H7 (a protein kinase inhibitor) into the prothoracic ganglion of previously selective females, resulted in the females' inability to discriminate between attractive and unattractive calls and were overall less responsive. However, nano-injection of membrane permeable cGMP into the prothoracic ganglion caused females to become more syllable period-selective than in the pre-tests. Nano-injection of saline into the prothoracic ganglion did not change the overall selectivity of the female's phonotaxis when compared to the pre-tests. These results suggest that circuits in the prothoracic ganglion can modulate syllable period-selectivity of phonotactically responsive females. Additionally, exposing virgin females to males, without permitting physical contact, caused a change in the number of syllable periods females typically respond to, when compared to the control virgin females. Females previously exposed to males were more likely to respond phonotactically to a single syllable period, while control virgin females responded to as many as five syllables periods. The effect of male exposure on the underlying circuits controlling female phonotaxis remains to be investigated as an additional modulators of female phonotaxis. Whether H7 and cGMP mediate their modulatory role by targeting similar or different targets remains to be investigated.

Poster Session 1 | Poster Wall 10 | Label: PS1.010

Category: Auditory system and acoustic signaling

The impact of early auditory exposure on vocalizations in a eusocial mammal

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Many organisms within the Animal Kingdom organize into large structured groups or societies. With this organization, there arises a need for effective communication across individuals. Vocal exchanges are used by many species as a method of communication. In humans and very few other taxa, these vocalizations are learned during development. Recently there has been compelling evidence that the naked mole-rat might also belong to this list of animals, as each colony uses a distinct vocal dialect which can be learned early on in life. To test whether the acoustic environment influences the development of naked mole-rat vocalizations, we raised pups in altered acoustic environments. After weaning, pups were removed from their home colonies and continuously exposed to either silence, a pure tone or a novel sound for six-months. Here we present results analyzing changes in acoustic features occurring in juveniles until six months of age. Our study suggests that the auditory environment during development influences naked mole-rat vocalizations.

Poster Session 1 | Poster Wall 11 | Label: PS1.011

Category: Auditory system and acoustic signaling

Resonant song recognition and the evolution of acoustic communication in crickets

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The function and evolution of calling song recognition networks is central to understanding speciation in insects. Evolutionary changes in song recognition networks can compel speciation, so understanding the commonalities in the mechanisms by which song recognition is achieved across species can help define a central or “mother” network architecture that is shared among closely related species in a group. Then, observed differences in phenotype across species can be related to changes within the shared network parameter space, to eventually understand how evolutionary mechanisms give rise to the diversity of observed behaviors. One instance of such a mother network for calling song recognition has been extensively characterized for the cricket species G. Bimaculatus. In crickets, females respond to certain features of male calling song, typically the interpulse period, within a limited range that defines a unimodal phenotype for calling song recognition. A computational model of this proposed network has been shown to recover all single-peaked recognition phenotypes known from crickets. This model consists of five neurons connected in a network with excitatory and inhibitory feedforward connections, adaptation, and linear filters. The novel, multi-peaked response of the Anurogryllus cricket presents a potential challenge to the mother network hypothesis. Here, we characterize this phenotype as resonant and examine the properties of such a resonance type alongside simplified mechanisms of song feature recognition with the aim of expanding the known capabilities of the mother network architecture, and exploring how resonance types may be selectively suppressed and recovered, allowing for fast transitions between tuning types in evolution.

Poster Session 1 | Poster Wall 12 | Label: PS1.012

Category: Auditory system and acoustic signaling

Inter-individual differences in behavioral and physiological responses under the stressful conditions : Quantitative assessment of personality in Japanese house bats

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Echolocating bats rely heavily on sound information, and their social calls contain important information. Distress calls (DCs), one of social calls, are used as a warning signal under stressful conditions. Although it is known that an animal's decision making can vary based on the static personality and dynamic emotions. However, previous studies in bats have not assessed inter-individual differences in the response of each individual under stressful conditions, i.e. personality. Thus, this study aimed to establish a quantitative assessment of personality by measuring physiological and behavioral responses. In this study, we measured the vocalization pattern of Japanese house bats' DCs and their direction of movement by gently stimulating them with a cotton swab. We investigated the relationship between physiological changes in heart rate and body temperature as autonomic nervous system activities related to emotion. The results showed that they mainly vocalized two types of DCs, frequency modulated (FM) and noise burst (NB) types. Furthermore, the intra-individual vocalizations rates remained consistently across periods. In particular, in females, the vocalization patterns were polarized to the NB-dominant and FM-dominant types. The FM-dominant vocalizers showed escape behavior from the cotton swab stimulation, while the NB-dominant vocalizers attacked to the swab. The results suggest that the vocalization pattern may be polarized due to the fight-or-flight response, suggesting that NB-dominant vocalizers are more aggressive than FM-dominant vocalizers. In addition, the NB-dominant vocalizers had greater increases in heart rate and body temperature than the FM-dominant vocalizers, suggesting inter-individual differences in sympathetic nervous system activity. In summary, bats have a consistent vocalization pattern even under same stressful conditions, and the inter-individual differences may be explained in autonomic nervous system response levels.

Poster Session 1 | Poster Wall 13 | Label: PS1.013

Category: Auditory system and acoustic signaling

Tools for the optical investigation of the naked mole-rat auditory cortex

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Naked mole-rats (Heterocephalus glaber) are highly cooperative, eusocial rodents, which live permanently underground in burrows in eastern Africa. They exhibit a rich vocal repertoire through which they transmit social signals to conspecifics. Their unique social structure and vocal behaviors make them particularly interesting for the study of the neural circuits underlying complex vocal communication behaviors. However, to date, few neuroscientific tools have been available to study thesee neural correlates in naked mole-rats.

To address this, we established a surgical protocol, and post-operative care protocol, for intracranial injections of AAV-viruses into the auditory cortex of naked mole-rats. We were able to successfully express both retrograde tracers and calcium indicators in naked mole-rat brains, with an expression time of 4-8 weeks. We further established a protocol for a cranial window implant, to allow optical access to the cortical surface for in vivo recordings. Using these newly developed surgical protocols, we performed two methods of imaging in awake, behaving naked mole-rats. First, we performed intrinsic signal imaging (ISI) to establish the positioning of the subareas of the auditory cortex in the naked mole-rat. Next, we performed two-photon imaging, determining the response of populations of excitatory auditory cortical neurons at a single-cell level to a variety of sound stimuli, including both pure tones and social stimuli.

Overall, we show that the naked mole-rat is amenable to surgical procedures required to perform advanced optical methods of neural recording. We establish a robust surgical procedure for intracranial viral injections and cranial window implantations, and show population level and single neuron level in vivo recordings of auditory cortical activity in the awake, behaving naked mole-rat.

Poster Session 1 | Poster Wall 14 | Label: PS1.014

Category: Auditory system and acoustic signaling

Mother bats respond to own pup's isolation calls with heart rate changes

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Isolation calls (ICs) are acoustic signals for pups' survival to attract parents' attention. In particular, ICs are significantly important for mother echolocating bats because they rely heavily on sound information for social communication. In addition, it is shown that acoustic features of ICs may play an important role in communication between mother and pups to identify own family members in their colony, and ICs selectively attract their own mother bats. In this scene, it is necessary to understand the internal processes of mother bats, the receiver of the vocalization, but the psychological effects of ICs to mother bats are unknown. In this study, we tested mother bats psycho-physiological response to ICs using acoustic playback and heart rate recording in Japanese house bats (Pipistrellus abramus). The results showed that the mothers showed a significantly higher increase in heart rate to their own pups' ICs rather than other pups. Comparing intra-individual changes over time, the increase in heart rate responded more highly to ICs closer day of the experiment than to the past ICs. In our previous study, a similar increase in heart rate was observed immediately after the presentation of the distress calls, possibly reflecting emotional changes via the autonomic nervous system when they heard social calls. Therefore, the present study suggests that the pups' ICs may selectively influence their own mother's internal state and supports the idea that ICs affect the recipient's emotions with the sympathetic nervous system dominance. We plan to investigate the acoustic features and selective mechanisms in future work.

Poster Session 1 | Poster Wall 15 | Label: PS1.015

Category: Auditory system and acoustic signaling

Crows can volitionally control the number of vocalizations produced

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A principal advantage of vocal communication is the ability to quickly and flexibly broadcast signals over long distances. The spectral and temporal characteristics of these signals are typically studied for their potential contained information but other features – such as the number of vocalizations produced – can also be informative of the milieu. For example, the number of repetitions of a chickadee alarm call note has been found to be correlated with the wingspan of the detected predator. However, whether this ability to produce differing numbers of calls is a consequence of the animal's fluctuating internal arousal state or under cognitive control is unknown. Here, we trained three carrion crows – corvid songbirds known for their numerical and vocal abilities, to produce differing numbers of calls to arbitrary stimuli demonstrating that this ability can be volitionally controlled. Task performance exhibited characteristics of the approximate number system such as numerical distance and magnitude effects. The time to vocal onset and the acoustic features of the first vocalization of a sequence were predictive of the total number of vocalizations, indicating a planning process that scales with the impending number of vocalizations. Finally, analysis of the vocal trajectories for correct and error trials also suggests that the crows might be 'keeping track' during production of these prepared vocal sequences, as acoustic features of each element could be used to read-out their ordinal position. Together, these findings illustrate the crows' remarkable ability to combine numerical competence and volitional vocal control as an important evolutionary precursor toward the advent of symbolic counting and communication.

Poster Session 1 | Poster Wall 16 | Label: PS1.016

Category: Auditory system and acoustic signaling

Auditory response to pup calls in California mouse fathers and virgin males

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California mice (Peromyscus californicus) are one of only ~5-10% of mammalian species in which fathers provide care for their offspring. Fathers in this species perform extensive paternal behavior and are highly attracted to both their own offspring and unfamiliar pups. In contrast, virgin males often ignore or attack pups. Moreover, fathers and virgins show different behavioral and neural responses to sensory stimuli from pups. In females, neural and behavioral effects of transitioning to motherhood have been studied extensively; however, the transition to fatherhood in males, without corresponding gestation and lactation, is not well understood. Here we present findings on differences in auditory responses to pup calls between experienced fathers and reproductively naive males. Using a 32-channel vertical array probe in the auditory cortex of urethane/xylazine-anesthetized California mice, we recorded responses to 100 ms noise bursts and 25 second pup calls presented repetitively. The corresponding local field potential recordings were transformed into current source density analysis to understand the temporal-spatial profiles of auditory response between virgins and fathers. Preliminary data indicate that the upper layers of the cortex have more stimulus-locked and stronger activity peaks in fathers compared to virgins. We hypothesize that fathers will have better inter-trial phase coherence across thalamic input layers, especially upper layers, due to higher valence of the pup call signal. We also hypothesize that the temporal resolution function will more accurately predict the stimulus from a model trained on fathers' auditory response than virgins due to higher attention. (Supported by NSF IOS-2118607.)

Poster Session 1 | Poster Wall 17 | Label: PS1.017

Category: Auditory system and acoustic signaling

Behavioural and physiological measures of hearing in a mutant mouse

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A spontaneous, recessive mutation in C57BL/6J laboratory mice (Mus musculus) resulted in offspring with an "Abnormally Wobbly Gait" (AWG). The AWG phenotype affected males and females equally and resulted in hyperactivity, continuous circling, and head-tossing behaviour. Despite abnormal locomotion, AWG mice ate, drank, and mated. Based on their phenotypes, AWG mice were suspected to have hearing deficits. We measured acoustically-evoked behaviour and hearing thresholds with acoustic startle response (ASR) and auditory brainstem response (ABR) recordings in AWG (AWG; n = 15), wild-type littermate control (WT; n = 9), heterozygous littermate control (HET; n = 11) and C57BL/6J control mice from a different colony (C57; n = 12). We recorded videos of ASRs in a foam-lined arena by presenting mice with a loud burst of white noise (107 dB SPL at 10 cm, 200 ms duration) three times. Raters (n = 19) watched videos and scored their confidence of an ASR on a 4-point Likert scale. We recorded ABRs with subdermal needle electrodes near the auditory bullae while stimulating mice with tones (8, 16, and 32 kHz; 5 ms) and/or clicks (0.1 ms) and averaging the evoked response (n = 512 trials). The maximum playback amplitude was 85 dB SPL. Recordings from C57, WT, and HET mice all showed characteristic ABR waveforms evoked by tones and clicks. Click-evoked thresholds for C57 mice (mean + SE: 46 + 0.8 dB SPL) were lower than WT (50 + 1.5 dB SPL) and HET mice (55 + 2.4 dB SPL). Most AWG mice did not show a clear ABR waveform with a detectable threshold. Average ASR scores for C57 and WT mice (C57 = 3.01; WT = 3.04) were higher than for HET (HET = 2.69) and AWG mice (AWG = 1.16). Indeed, most AWG mice did not exhibit an ASR. Intraclass correlations showed ASR scoring was consistent across raters (ICC = 0.732; F(119,1042) = 58.6, p

Poster Session 1 | Poster Wall 18 | Label: PS1.018

Category: Auditory system and acoustic signaling

Hearing capability in actively behaving bats: Effects of experimental methods and detection tasks

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Echolocating bats are nocturnal mammals that rely heavily on acoustic signals for communication and spatial orientation. Unlike the passively receiving of environmental information by sensory systems such as vision, spatial information obtained by many bats is through an active process called echolocation. For echolocating bats, self-produced calls can interfere with the auditory processing of following echoes that are typically much weaker than the emitted calls, especially for those that produce constant-frequency (CF) sonar signals. However, auditory detection ability under behavioral states in CF bats remains poorly understood. In this work, we compared the behavioral and physiological audiograms of a CF bat species, Hipposideros pratti, using a 2-AFC psychophysical setup and evoked potential recordings of the middle brain inferior colliculus. We showed that both testing methods and detection tasks affected the hearing sensitivity measurements in bats. First, we found marked differences in both audiogram shape and absolute thresholds between the behavioral and neurophysiological methods. Second, we found that the effects of temporal integration on the behavioral detection thresholds depended on the signal frequency, while evoked potentials did not show evidence. Third, we measured hearing sensitivity in different tasks to explore the potential effect of vocal production: a passive listening (PL) task to detect pure tones, an active listening (AL) task to detect pure tones triggered by self-emitted vocalization, and a phantom echo task. We found that H. pratti had excellent hearing sensitivity (approximately 0 dB SPL) in the PL task but performed nearly 40 dB worse in the AL task and the echo task. In the AL task, all bats gradually increased call frequency by 0.8 to 1.1 kHz to overcome self-generated auditory masking. These results stress the need to study the hearing capability of bats engaged in natural behavioral tasks, which is yet seriously under-explored.

Poster Session 1 | Poster Wall 19 | Label: PS1.019

Category: Auditory system and acoustic signaling

Prevalent harmonic interaction in the bat inferior colliculus

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Many human speech and animal vocalizations are characterized by a complex spectral-temporal structure, composed by multiple harmonics, and manifested as dynamic sequences. However, auditory research often uses simple artificial acoustic stimuli or their combinations to probe the auditory system. How does the inferior colliculus (IC), the first integration center in the ascending auditory pathway, process harmonic information within a natural sequence context? We recorded extracellular single-unit activity of IC neurons in anesthetized Hipposideros armiger using natural echolocation sequences and manipulated sequences of incomplete vocalizations. We report that approximately two-thirds of IC neurons exhibited harmonic interaction. Neurons with high harmonic interactions exhibited greater temporal selectivity to natural echolocation sequences, and the degree of harmonic interactions is virtually unaffected by the amplitude relationship between the harmonics. Meanwhile, for 85% of the IC neurons, the responses to natural echolocation sequences could not be predicted by manipulated sequences of incomplete vocalizations. Surprisingly, nearly 40% of the neurons that showed a harmonic interaction were characterized by a single excitatory response peak as measured by pure tones. Our results suggest that prevalent harmonic processing has already emerged in the auditory midbrain inferior colliculus in the echolocating bat.

Poster Session 1 | Poster Wall 20 | Label: PS1.020

Category: Auditory system and acoustic signaling

The Distorted Hearing of Mosquitoes: the biophysical bases of an unconventional ear

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Hearing, as experienced by humans and vertebrates, relies heavily on an active process – a unique mechanism for the amplification, and frequency discrimination, of sound. A poorly understood feature of this active process are self-sustained oscillations (SSOs). Here, the flagellar ears of mosquitoes, being one of the most sensitive biological sensors in the invertebrate kingdom, have emerged as ideal model for these phenomena.

In mosquitoes, SSOs are male-specific, naturally occurring and believed to be crucial for locating a mate within a swarm, they bear resemblance to spontaneous otoacoustic emissions (SOAEs) in hair cells of vertebrate inner ears but exceed these by orders of magnitude in their energy investment.

Recent research reveals an intricate system designed to solve a perplexing auditory issue: It all starts with a mismatch between the female's wingbeat frequency and the frequency response function of the male's auditory nerve. The mosquito resolves this discrepancy using complex, distortion-product (DP) based tones, creating audibility from 'ultrasound'. The required nonlinearities can be traced back to the mechanotransducer level.

Concomitant analyses of flagellar motions and auditory nerve responses suggests that not all nerve DPs arise from flagellar distortions, hinting at more complex, multi-layered nonlinear mechanisms. Furthermore, SSOs vastly transform the auditory landscape providing exquisite tonal richness without compromising frequency selectivity.

Interestingly, our work shows that the male auditory system can distinguish between pure tones and DPs with the latter enjoying a sensitivity threshold ten times lower than their pure tone counterparts.

These findings open a promising path to modelling (also human) audition by shedding light on the link between SSOs and hearing loss, potentially inspiring new generations of DP-based hearing aids.

Poster Session 1 | Poster Wall 21 | Label: PS1.021

Category: Auditory system and acoustic signaling

Neural Discrimination of Vocalization in the Auditory Cortex of the Mexican Free-tailed Bat

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We investigated the neural basis of auditory processing for communication calls in the Mexican free-tailed bat, Tadarida brasiliensis, a species known for its complex and highly elaborate vocal repertoire. We addressed the question of whether the encoding of conspecific call types depends on the response selectivity of individual neurons or the collective response of multiple neurons within a structured layer organization in the primary auditory cortex. We analyzed the selectivity and decoding properties and layer organization for neurons in response to ten types of communication calls and two types of sonar pulses as acoustic stimuli. We found that neurons in the primary auditory cortex exhibit a wide range of selectivity to these social calls, indicating a diverse neural tuning. Moreover, we identified collective processing mechanisms in the auditory neurons for decoding call types. This was achieved using supervised classification based on the neuron's firing rate relative to the baseline activity (measured as the Z score) and temporal components defined by the interspike intervals (ISI) of the neuron's action potentials. This approach provided an understanding of how auditory neurons process and differentiate between different call types, emphasizing the importance of both the rate and timing of neuronal firing in the auditory decoding process. This mechanism indicates that the distinction among communication calls relies on a neural ensemble capable of modulating their response to a spectrum of sound features across different cortical layers. Our findings contribute to a deeper understanding of the neural mechanisms underlying auditory processing in the Tadarida brasiliensis bat model, highlighting the complexity of neural encoding in bat communication.

Poster Session 1 | Poster Wall 22 | Label: PS1.022

Category: Auditory system and acoustic signaling

Cortical representation of social sounds in the naked mole-rat

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The naked mole-rat (Heterocephalus glaber) is a subterranean rodent that forms highly cooperative, hierarchical colonies headed by a queen. These social mammals also produce over 25 distinct vocalizations that encode individual and colony identity, thus representing a promising and unexplored model to study the neural circuits underlying social communication. To investigate the network dynamics of auditory cortical processing in response to social sounds we are performing in vivo electrophysiological recordings in the auditory cortex during natural and synthetic sound presentation. We expect to find highly localised brain responses to conspecific sounds, reflecting specialized vocalization-mediated social recognition, as well as differential activity depending on the social rank of the conspecific vocalization that is heard. Here we present the first results of our in vivo studies including the development of methodology for in vivo recordings in the naked mole-rat brain. We also present a preliminary characterization of the tonotopic organization of the auditory regions in the naked mole-rat brain with a particular focus on regions enhanced in the processing of conspecific vocalizations.

Poster Session 1 | Poster Wall 23 | Label: PS1.023

Category: Auditory system and acoustic signaling

Does Inferior Colliculus neurons underlie behavioral auditory discrimination in noise?

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Which structures of the auditory system are involved in the ability to discriminate target stimuli in background noise? Over the last decades, a large number of studies have described the robustness of auditory cortex responses when target stimuli are presented in situations of acoustic degradation, which are common to our everyday lives. (Nagarajan et al.2002, Mesgarani et al. 2014, Town et al, 2018). In few experiments, comparisons between cortical and subcortical neurons recording in noise have been made. (Schneider and Woolley.2013, Rabinowitz et al .2013). While several studies claimed that the behavioural discrimination performance correlates with the auditory cortex neuronal responses, we reported that in anaesthetized guinea pigs inferior colliculus neurons responses showed the highest resistance to noise compared to the other auditory structures neurons. (Souffi et al 2020, 2023). To determine if subcortical neuronal discrimination could be correlated with behavioral auditory discrimination, we trained mice in a Go/No-Go protocol to discriminate between two hetero-specific vocalizations (one CS+ and one CS-) first in silence and then in increasing levels of stationary noise and then of chorus noise. Neuronal recordings were collected in the inferior colliculus of these mice, both in anesthetized and in awake, passively listening conditions. We compared these data with those from two control groups. In the first one, animals were similarly and passively exposed to the stimuli with no behavioral task. In the second group, animals were never exposed to the stimuli. Those data should allow us to determine if neuronal responses from the inferior colliculus can predict the behavioral performances of an animal and understand better the implication of the inferior colliculus neurons in stimuli discrimination in noisy background.

Poster Session 1 | Poster Wall 24 | Label: PS1.024

Category: Auditory system and acoustic signaling

Categorization and laminar organization of spectrotemporal receptive fields in auditory cortex of free-tailed bats

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Many species of echolocating bats can resolve fine texture of objects by emitting downward frequency-modulated (dFM) pulses and then analyzing the pattern of spectral notches in returning echoes. Texture perception likely requires more fine-grained echo-acoustic processing than simple frequency perception does, and therefore should involve more complex patterns of neural activity and recruit relatively more neurons from superficial layers 2/3 of auditory cortex. To test this hypothesis indirectly, we stimulated free-tailed bats (T. brasiliensis) with dynamic moving acoustic ripples and examined the complexity and laminar distribution of resultant spectrotemporal fields (STRFs) in the primary auditory cortex. We hypothesized that superficial layers 2/3 would produce STRFs with temporally stacked alternating excitatory and inhibitory subfields, a pattern thought to mediate more complex auditory processing like that in echo-acoustic texture perception. Clustering solutions differed by analysis type, but the most important factors appeared to be number of excitatory peaks as well as the frequency bandwidth and stacking of subfields. STRFs of cells in layer 2/3 showed more stacked STRF subfields than layer 4 did, supporting our hypothesis that more complex auditory processing occurs in these superficial layers. This study is also the first known examination of STRFs in bats, and the clarity of receptive fields produced in our study supports the use of bats for STRF research.

Poster Session 1 | Poster Wall 25 | Label: PS1.025

Category: Auditory system and acoustic signaling

Impact of sub-lethal dosages of the insecticides imidacloprid and flupyradifurone on ascending auditory interneurons in the cricket brain

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Insecticides are an essential part of modern agriculture. However, their extensive use has also been associated with the world-wide decline of the insect fauna over the last decades. Therefore, it is crucial to better understand the impact of modern insecticides on non-target insects with diverse behavioural lifestyles. Here, we analysed the effect of the classical neonicotinoid imidacloprid and the more recently marketed flupyradifurone on the auditory pathway of the field cricket Gryllus bimaculatus. Both insecticides act as highly selective agonists at achetylcholine receptors in the insect CNS. We recorded the spiking activity of ascending auditory interneurons AN1 and AN2 using a suction electrode at the brain surface. We measured the spiking response to acoustic stimulation (5, 10, 15, 20 and 30 kHz at 70, 75 and 80 dB) while applying different concentrations (10^-6, 10^-5, 10^-4 mol/l) of the respective insecticide. Both insecticides caused a similar dose-dependent reduction of spiking responses in both ascending auditory interneurons. Crickets rely heavily on auditory information for phonotactic mate finding and predator avoidance. Therefore, the disruption of auditory processing may substantially reduce their chances for survival and reproduction.

Poster Session 1 | Poster Wall 26 | Label: PS1.026

Category: Auditory system and acoustic signaling

Female Cope’s gray treefrogs change movement patterns, but not preferences, when facing a more complex discrimination task

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In animal communications, receivers must identify which signals are relevant, where they are coming from, and what response to make. Tracking a series of signals from a single source over time can be difficult, especially in complex environments when competing signals are similar. Here, we sought to understand how behavioral patterns when approaching salient signals are altered in the presence of competing signals in Cope’s gray treefrogs (Hyla chrysoscelis). Like many frog species, these females display phonotaxis towards males in ponds where many conspecific and heterospecific males call at once. Leveraging the strong preferences females display for conspecific calls of longer duration, we measured how different types of unattractive competing calls change the patterns of movement during phonotaxis. We used playback experiments with an accelerometer/gyroscope to measure movements with high sensitivity and temporal resolution in gravid females. Females were surrounded by four speakers in an anechoic chamber and presented with stimuli in which one speaker broadcast a series of attractive calls and three speakers broadcast unattractive ‘distractor’ calls that were either conspecific (same pulse rate, half the duration) or heterospecific (half the pulse rate, same duration) in alternation. Trials that received heterospecific distractors had a high likelihood and strength of movement within 500ms after an attractive call and no movement after the distractor. However, conspecific distractors elicited an equal likelihood and strength of movement compared to movement following the attractive call. Despite these differences, trials with heterospecific and conspecific distractors did not differ in phonotaxis latency to the attractive call. In sum, movement patterns changed when more salient conspecific distractors were present, suggesting alternative strategies for decision making in complex signaling environments.

Poster Session 1 | Poster Wall 27 | Label: PS1.027

Category: Auditory system and acoustic signaling

Auditory competition in owl midbrain space maps

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The natural environment challenges the brain to prioritize the processing of salient stimuli. The barn owl, a sound localization specialist, exhibits a circuit called the midbrain stimulus selection network, dedicated to representing locations of the most salient stimulus in circumstances of concurrent stimuli. Previous competition studies using unimodal (visual) and bimodal (visual and auditory) stimuli have shown that relative strength is encoded in the spike response rates. However, open questions remain concerning competition between concurrent auditory signals on coding. To this end, we presented diverse auditory competitors (concurrent flat noise and amplitude modulated noise) and recorded neural responses of awake barn owls in subsequent midbrain space maps, the external nucleus of the inferior colliculus (ICx) and optic tectum (OT). While both ICx and OT exhibit a topographic map of auditory space, OT also integrates visual input and is part of the global-inhibitory midbrain stimulus selection network. Through comparative investigation of these regions, we show that while increasing strength of a competitor sound decreases spike response rates of spatially distant neurons in both regions, relative strength determines spike train synchrony of nearby units only in OT. Furthermore, changes in synchrony by sound competition in OT are correlated to gamma range oscillations of local field potentials (LFPs), associated with input from the midbrain stimulus selection network. The results of this investigation suggest that modulations in spiking synchrony between units by gamma oscillations are an emergent coding scheme representing relative strength of concurrent stimuli, which may have relevant implications for downstream read out.

Poster Session 1 | Poster Wall 28 | Label: PS1.028

Category: Auditory system and acoustic signaling

Sensing in the swarm: spectro-temporal variation may facilitate self-recognition of echoes for bats flying in dense groups

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Most biosonar models predict that bats rely on the correct assignment of an echo from a broadcast signal to successfully perceive their environment, which should be difficult for bats in dense groups. Brazilian free-tailed bats (Tadarida brasiliensis) form some of the largest aggregations on the planet and have flexible characteristics of their echolocation signals. We hypothesize they use subtle variations in spectro-temporal characteristics to facilitate echolocation in dense groups. To record from inside the swarm, we used both a stationary microphone that opportunistically captured the passing bat swarm, and a custom video and acoustic recorder carried by a trained hawk that flew through the swarm. We computed spectrograms from the acoustic recordings and extracted “time-frequency ridges”. These ridges were fit to low-order polynomials to generate model frequency modulation functions, which in turn uniquely determine continuous time-series representations of modeled emission waveforms. Standard signal detection methods (cross correlation and background normalization) enabled quantitative estimation of detection performance, including rejection of interfering emissions and echoes. Our results demonstrate that subtle but specific variation in spectro-temporal shape can constitute the basis of call differentiation, which may be an adaptive strategy used to reject acoustic signals from conspecifics when echolocating in dense swarms.

Poster Session 1 | Poster Wall 29 | Label: PS1.029

Category: Auditory system and acoustic signaling

Context-dependent Vocalisations in Laboratory Mice

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Laboratory mice are a relevant example in neuroethology to understand context-dependent vocal signalling and adaptive behaviour mechanisms. Mice are known to vocalize more frequently in social contexts, yet research focusing on the interaction between vocal production and reception during natural behaviours such as courtship calls remains limited. The influence of context on the production of vocalizations remains poorly studied. Vocalizations in mice are highly individual, exhibit developmental variation, and are influenced by social housing conditions. Here we examined the impact of the presence of female mice and their oestrous state on male mice vocalizations by investigating their responses to female urine scent cues. Our study involved six wild-type laboratory mice (three female and three male) and eight transgenic APPPS1 AD mice (five female and three male). Additionally, we explored correlations between vocalizations emitted by female mice in response to male urine scent cues under both Wild-type and Alzheimer's conditions. Our results revealed variations in vocalizations among male mice, with males producing more vocalizations compared to females when exposed to urine cues. Furthermore, we observed that male mice vocalizations were influenced by the oestrous phase of their female counterparts. These findings indicate the sensitivity of mouse courtship vocalizations to changes in female oestrous phase and scent cues, highlighting the context-dependent nature of these vocal signals.

Poster Session 1 | Poster Wall 30 | Label: PS1.030

Category: Auditory system and acoustic signaling

Neural coding underlying perception of auditory phantom signals

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Proper sound localization plays a quintessential role in survival across species, enabling vital environment-dependent mechanisms such as hunting, mating, and fleeing from predators. Barn owls (Tyto alba) have evolved expertise in sound localization, where binaural cues of interaural time difference (ITD) along the horizontal plane (azimuth) and interaural level difference (ILD) along the vertical plane (elevation) create a topographic map of space in the midbrain. Localization success increases with sound bandwidth, and barn owls perceive pure tones as spatially ambiguous sounds. When a pure tone originates from a single location, the owl perceives multiple ITDs. The perceived-but-false sound sources in response to pure tones are known as phantom sound sources. Behavioral studies demonstrated that owls head-turn towards both true and phantom sound sources, but successfully localize the true source when the signal's bandwidth exceeds 3kHz. Consistent with behavioral results, recordings in the optic tectum (OT, part of the midbrain) demonstrated tuning curve changes with increased side peak suppression when the signal's bandwidth exceeded 3kHz. However, these were single neuron recordings, and pure tones result in activation of multiple neuronal populations across the auditory map. Additionally, when presented with multiple sound sources, the midbrain stimulus selection network globally suppresses responses of neurons whose preferred ITD, and possibly frequency, characteristics are different from those of the strongest stimulus. Thus, we propose using a multi-electrode array (MEA) to concurrently observe pure tone responses of neurons across the entire OT map, and hypothesize suppression of responses at non-preferred ITDs and frequencies by the midbrain stimulus selection network.

Poster Session 1 | Poster Wall 31 | Label: PS1.031

Category: Auditory system and acoustic signaling

Social dynamics modulate the patterning of male calling behavior in a South American treefrog

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Animals communicate in diverse environments using complex sequences of acoustic signals. In anuran species, vocalizations function to attract females, repel potential competitors, and establish territory boundaries between neighbors. Male frogs adjust their call patterning depending on the environment, ambient noise, and their own body size. Understanding the patterning of calling behavior provides insight into the evolution of acoustic communication across systems. Here, we examine how social feedback affects the temporal patterning of male calls in the South American treefrog, Boana pulchellus. We analyzed how males modulate their call patterning in response to acoustic playbacks that varied in complexity. We find that males adjust the variability in their call type repertoire as well as in their call sequencing depending on stimulus complexity. Taken together, these results suggest that males rapidly assess complexity in acoustic stimuli and modulate their vocalizations according to these social inputs, highlighting the flexibility of this species' vocal behavior. This flexibility may be a result of the neural processing plasticity associated with emitting communicative signals.

Poster Session 1 | Poster Wall 32 | Label: PS1.032

Category: Auditory system and acoustic signaling

Distinct activity patterns in auditory cortex underlie echolocation and communication calls in bats

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Acoustic communication is of critical importance in a large number of vertebrates. The bat Carollia perspicillata is a highly social animal and emits sounds using a rich vocal repertoire which can be broadly classified into echolocation and communication calls. Yet to date, neuronal mechanisms underlying these vocalizations are not fully understood. Previous studies indicate that the auditory cortex not only plays a role in sound perception, but also in the preparation and processing of self-produced sounds. To identify and characterize potential activity patterns during call emission, we performed neuronal recordings in the auditory cortex of C. perspicillata while they engaged in spontaneous vocalizations. When we analyzed spiking and local field potentials across cortical layers, we found distinct activity patterns related to the type of call the animal was emitting. Additionally, spiking strength at call onset differed depending on whether the bat was producing a single call or a sequence of vocalizations. These results add to the current understanding of neuronal mechanisms underlying vocal control and may have broader implications for other species with extensive vocalization abilities.

Poster Session 1 | Poster Wall 33 | Label: PS1.033

Category: Auditory system and acoustic signaling

Separation Slang – Uncovering separation induced low frequency mouse vocalizations

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Direct physical interaction constitutes a fundamental component of social engagement across various mammalian species. When such interaction is restricted in mice, specific separation-induced social communication emerges. Here, we separated age-matched mice of same and opposite sex from each other using a transparent Plexiglas divider containing a porous section at its bottom. Following this separation phase, the divider was removed, and mice were allowed to freely interact with one another. Audio and video were recorded during both the separation and unification phases. Separated same-sex mice emitted a set of vocalizations distinct from previously reported ultrasonic vocalizations (USVs), referred to as separation-induced calls (SICs), while during unification mostly conventional USVs were emitted. Notably, approximately 70% of SICs exhibited peak frequencies ranging from 6 to 46 kHz, with three distinct modes at 6-8 kHz, 16-18 kHz, and 36-38 kHz – significantly lower than conventional USVs, which resulted in the categorization of these calls into low and middle frequency vocalizations. In contrast to USVs, only about 50% of SICs occurred within a bout structure. Within bouts, inter-call-intervals were similar for SICs and USVs. Contrary to same-sex pairs, separating opposite-sex pairs led to a significantly higher production of established USVs (75%).

Our study highlights the remarkably consistent production of vocalizations at substantially lower frequencies than previously described, emitted by mice when subjected to separation. Notably, such vocalizations have so far only been documented in highly aversive circumstances, such as restraint.

Poster Session 1 | Poster Wall 34 | Label: PS1.034

Category: Auditory system and acoustic signaling

Development of sound production in Danionella cerebrum

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Acoustic signaling, integral to intraspecific communication and reproductive behavior, undergoes notable changes during an animal's ontogenetic development. The onset and progression of this maturation in fish remains poorly understood. Here, we investigate the ontogeny of acoustic communication in the micro glassfish Danionella cerebrum (DC), one of the smallest known vertebrates and an emerging model organism in neuroscience. Adult DC males produce audible clicks that appear in sequences with a repetition rate of 60 or 120 Hz, caused by consecutive unilateral or alternating bilateral compressions, respectively. To investigate the maturation of this ability, we performed long-term sound recordings and morphological studies of the DC sound production apparatus throughout its ontogenetic development. We found that DC start producing clicks during the second month of their lives and continually increase their abundance and structured repetition over the course of the following one to two months. The sound production machinery, including specialized bone and cartilage structures, start to form in male DC after approximately four weeks and prior to full maturation of the reproductive organs. While the DC clicks increase in amplitude with age and body size, click repetition rates of 60 and 120 Hz are stable throughout development. This suggests a fully developed central pattern generator in juvenile males, yet a continued maturation of the drumming apparatus capable of creating louder sounds.

Poster Session 1 | Poster Wall 35 | Label: PS1.035

Category: Auditory system and acoustic signaling

Representation of sounds in the cortex of naked mole-rats

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Naked mole-rats are small rodents with very elaborate colony organization with up to 300 members. Their colonies structure is highly hierarchical and cooperative. Naked mole-rats have a rich vocal repertoire that consists of ~25 vocalization types. The most commonly used vocalizations are the so-called soft chirps (SC). SC are used antiphonally with repeated call and response bouts between colony mates. Acoustic playbacks of SC elicit vocal responses in receiver animals, only if the SC belong to a member of the same colony as the listener. This behavior requires an exquisite discrimination of the acoustics of SC. Although machine learning classifiers showed that SC encode information about colony membership, it is unknown how these vocalizations are encoded in the brain of these animals. Sensory cortices are particularly plastic compared with lower system levels. The functional structure of cortex is continuously modified by an animal's sensory experience. Considering this, we hypothesize that a fine discrimination of colony SC takes place in cortical neurons. To answer this, we perform in vivo electrophysiology recordings across cortical layers and quantitatively evaluate physiological responses to natural vocalizations and to synthetic sounds. We expect that SC will be represented differentially in the cortex compared to other sounds that are acoustically similar but rarely encountered in the life of these animals.

Poster Session 1 | Poster Wall 36 | Label: PS1.036

Category: Auditory system and acoustic signaling

Segmenting variable animal behavior with vocal signals and machine learning

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Mice produce ultrasonic vocalizations during many social interactions. What behaviors cause or are elicited by these ultrasonic vocalizations is unclear because both the signals and the associated behaviors are quite variable. We hypothesized that the relationship between variable signals and variable behaviors might be hard for human observers to discover but might be discoverable using machine learning. Typically, supervised machine learning classifiers of animal behavior require extensive human-labeled training datasets, but here the vocalizations themselves can serve as the labels, as they are produced by animals precisely at the time of relevant behavior. We made audio and video recordings of interacting male and female mice, tracked the mice from the video, and trained a classifier using vocalization timing as labels and features extracted from the trajectories of the mice. If it is possible to train a "vocalization" classifier using video features, then information about vocalizations is present in the video frames, and thus in the behavior of the animals. We trained a classifier using JAABA (Kabra et al, 2013) and found it performed extremely well (precision: 85.2%, recall: 93.9%), suggesting that information about vocalizations is available in the pose and movement of the mice. What aspects of mouse social interaction are correlated with vocal signals? We took advantage of the structure of the classifier itself, and clustered video sequences using a distance metric determined by the classifier. This clustering revealed multiple, distinct behaviors associated with vocalizations. Once a putative behavior is identified, a new classifier can be trained by supervised machine learning and evaluated against the extrinsic signal. This method, for interactively exploring a classifier generated by extrinsic labels, may be extended to other systems to explore variable behavioral responses.

Poster Session 1 | Poster Wall 37 | Label: PS1.037

Category: Auditory system and acoustic signaling

Three-Dimensional Hearing in the Parasitoid Fly, Ormia ochracea

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The parasitoid fly, Ormia ochracea, eavesdrops on calling field crickets to use as hosts. Specialized tympanal hearing in O. ochracea supports accurate localization of cricket songs, by which female flies phonotactically approach hosts to deposit their parasitic larvae. In this paper, we examine the behaviour of female flies during larviposition (ie in close proximity to a sound source, following phonotaxis) to identify the role of acoustic cues at this stage of fly-host interaction. We show that flies are able to project larvae from distances up to several cm, by aiming at the sound source, and are sensitive to azimuth, elevation, and distance of the source. Flies are thus capable of localizing a sound source in three dimensions, although their auditory directional mechanism is only known to function in azimuth discrimination. Our data show that flies use a behavioural active-scanning strategy to derive information on source elevation.

Poster Session 1 | Poster Wall 38 | Label: PS1.038

Category: Auditory system and acoustic signaling

Inter-individual differences in linked vocal phenotypes

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Humans often show consistent individual differences in motor skills. Although it is increasingly appreciated that phenotypes are organized as suites of behaviors that change together, the degree to which the genetic variation underlying one behavioral phenotype influences other distinct phenotypes (pleiotropy) is unclear. Here, we use the Bengalese finch (Lonchura striata domestica) to test whether multiple motor behaviors exhibit linked variation across individuals. Finches have been widely studied because they learn to produce complex and individually distinctive songs, with parallels to human speech learning. These birds also produce other vocalizations that occur across different timescales and social contexts, such as simpler calls used in precisely timed vocal exchanges resembling conversations. We tested whether individual differences in performance are correlated across these distinct vocal behaviors. We found that birds with faster songs also generate faster trains of calls and respond more quickly during vocal turn-taking. Cross-fostering experiments show that call timing, like song learning, is shaped by the interaction of experience and genes. Using manipulations of tutoring experience known to alter the influence of genes and environment on song learning we demonstrate that these vocal phenotypes are dissociable. Thus, even if these phenotypes are influenced by shared neural and genetic mechanisms, social experience can independently shape each phenotype. To investigate differences in gene expression that might contribute to these inter-individual differences, we used bulk RNA-seq in vocal control regions. We found multiple genes associated with GABAergic signaling with increases expression in fast birds. Finally, spatial transcriptomics revealed correlated changes in non-vocal regions suggesting the genes linked with multiple vocal phenotypes may be more broadly pleiotropic and that heritable contributions to vocalizations shape other motor phenotypes.

Poster Session 1 | Poster Wall 39 | Label: PS1.039

Category: Auditory system and acoustic signaling

Investigating Vocal Neurons in Xenopus laevis and Silurana tropicalis

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Clawed frogs communicate acoustically to coordinate reproduction. In two closely related species, Xenopus laevis and Silurana tropicalis, males produce species-specific advertisement calls to attract females. Male advertisement calls of both species share slow trills containing clicks repeated at 30Hz. However, only the calls of X. laevis contain trills with clicks repeated at ~70 Hz. Interestingly, we previously found that the parabrachial nucleus, a premotor nucleus, is active only during fast trills and contains neurons called Fast Trill Neurons (FTNs) that drive the laryngeal motoneurons during fast trills. Here, we hypothesized that FTNs are only found in species that produce fast trills and are therefore absent in S. tropicalis. To test this hypothesis, we utilized “constellation pharmacology” (a technique to identify the receptors and ion channels expressed by dissociated neurons by monitoring intracellular Ca2+ levels in response to the application of agonists/antagonists) to characterize molecular profiles of the neurons in the parabrachial nucleus of the two species. Unexpectedly, we found putative FTNs in S. tropicalis, defined by the co-expression of GABA and NMDA receptors. Further investigation, however, revealed that the frequency of neurons with fast-kinetic voltage-gated K-channels is higher in X. laevis than in S. tropicalis, suggesting that the firing rates of the putative FTNs of male X. laevis is higher than those of S. tropicalis. To date, we successfully carried out single-cell RNAseq on the parabrachial neurons of male X. laevis, which allowed us to characterize the mRNA expression patterns. Extending these analyses to the parabrachial neurons of male S. tropicalis will likely provide us with a more detailed understanding of the molecular differentiation of homologous neurons in the two species, leading us to predict the functional divergence of the neurons through evolution.

Poster Session 1 | Poster Wall 40 | Label: PS1.040

Category: Auditory system and acoustic signaling

Model-based exploration of sensory constraints on grasshopper song recognition and speciation

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Many animals attract potential mates by acoustic courtship signals, or songs. Speciation requires a co-evolution of both song production and perception. How do sensory representations of songs influence song production in the context of speciation events? The auditory system of grasshoppers is a simple sensory pathway that mainly serves to recognize conspecific songs. Based on what is known about the system's physiology, we developed a grasshopper song detector model in order to explore constraints for species-specific song recognition that act on both the system and the signals.

The first processing stages of the model extract and logarithmically transform the amplitude modulation of an acoustic input signal into an intensity-invariant representation. This signal is expanded into a high-dimensional representation by convolutions with a set of Gabor kernels. A threshold non-linearity followed by temporal averaging is applied to each kernel response to create a set of slowly changing features, which uniquely characterize the songs of a given species. A perceptron adjusts the weights of individual features and thereby learns to detect its target species. We are currently using songs of more than 20 European Gomphocerinae species to test the detection performance of this model.

With this physiologically motivated but sufficiently reduced model of a grasshopper's auditory system, we are able to explore how the overall structure and specific parameters of this sensory pathway constrain the detectability of different grasshopper songs. For example, how much and in what way does the choice of kernels influence song discriminability? Is speciation constrained by this specific sensory representation, i.e. do distances between songs in feature space explain phylogenetic distances? These questions are difficult to answer with electrophysiological experiments. Our model-based approach allows to explore the optimal design of a specific sensory pathway in an evolutionary context.

Poster Session 1 | Poster Wall 41 | Label: PS1.041

Category: Auditory system and acoustic signaling

Masking of signals by both spectrally overlapping and non-overlapping noise impairs vibrational communication in a plant-dwelling insect

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The issue of noise is ubiquitous across sensory systems, with most research conducted in auditory systems, where noise directly affects communication by impairing signal perception at the level of sensory organs due to signal and noise interference or in the nervous system due to signal processing (termed energetic and informational masking, respectively). Communication via substrate-borne vibrations plays a crucial role in the reproductive success and survival of many insects, however, the influence of noise on it remains inadequately understood. We investigated the influence of band-pass noise of different spectral compositions on vibrational communication of the bug Nezara viridula (Hemiptera: Pentatomidae), revealing its negative effects both when overlapping and when outside the signals' frequency range. Our findings challenge the conventional view from acoustics, which limits masking only to cases of spectrally overlapping noise. We found that all noise types impaired the ability of males to recognize the female calling song (FCS) and localize its source. The noise decreased both the response (spike rate) of phase-locking sensory cells to FCS as well as disrupted the spike-time encoded frequency of FCS. Analysis of substrate vibrations, mimicking spike triggering in a sensory cell, showed that the disruption is caused by the interference that modifies the FCS waveform period. Noise also interfered with the time delays between signal arrival to different legs, which serve as the sole reliable cue for source localization. Our study is the first to demonstrate energetic masking by spectrally non-overlapping noise in a mechanosensory system. It highlights the vulnerability of vibration-mediated behaviour to noise disturbances, with implications for insect communication in natural and anthropogenically altered environments.

Poster Session 1 | Poster Wall 42 | Label: PS1.042

Category: Auditory system and acoustic signaling

Neural and behavioral evolution in an eavesdropper with a rapidly evolving host

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The diversification of animal communication systems is driven by the interacting effects of signalers, intended receivers, unintended receivers, and the environment. Yet, the critical role of unintended receivers like eavesdropping enemies in signal evolution has received little attention. Furthermore, the contemporary evolution of novel animal signals is rare, making it difficult to directly observe the role of unintended receivers in this process. Female parasitoid flies of the genus Ormia are obligate parasitoids and rely exclusively on acoustic cues to locate singing male orthopterans. In Hawaii, selection imposed by Ormia ochracea has led to recent and rapid phenotypic diversification of new songs in their local cricket host, Teleogryllus oceanicus. We use a complementary set of lab and field experiments to compare the neural and behavioral responses of Hawaiian flies to those of an ancestral fly population to understand the role of receiver psychology in the evolution of novel host songs. We demonstrate that introduced Hawaiian flies have evolved differences in neural auditory tuning and behavior that likely facilitate the detection of novel host songs. Hawaiian flies prefer novel song variants with certain characteristics, enabling us to make predictions about how selection imposed by the fly may shape song features in the future. Our findings provide a rare example of rapid evolution in the sensory tuning of an eavesdropper to correspond with a rapidly evolving host.

Poster Session 1 | Poster Wall 43 | Label: PS1.043

Category: Auditory system and acoustic signaling

Non-imitative vocal production learning in seals

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Vocal production learning (VPL) is the ability to produce novel or modify existing vocalizations by listening to acoustic signals. Learned vocalizations differ from the innate repertoire, such as a non-human animal (e.g., a parrot) imitating human speech. VPL is rare among mammals, but both grey (Halichoerus grypus) and harbor seals (Phoca vitulina) vocally learn through imitation. It remains unknown whether seals can also vocally learn based on non-imitative mechanisms, as recently shown in zebra finches (Taeniopygia guttata). Through an innovative experimental approach, we studied VPL guided by non-auditory feedback in three harbor seals and one grey seal. The animals were tested for their ability to modify vocal parameters associated with different levels of the sound production system, namely duration (respiration), fundamental frequency (f0; phonation), and center of gravity (CoG; vocal filter). The seals were trained to modify their vocalizations so that these parameters were altered in one direction (up or down). The experiment started with an initial threshold of the seals' pre-experimental median parameter (duration/f0/cog) distribution. Upon reaching the learning criterion (2 consecutive sessions with ≥ 80% correct responses) the threshold for a successful trial was increased, gradually shaping the seal's calls in the desired direction. A custom-made application enabled the direct evaluation of the call parameters' increase or decrease during the experiment, facilitating immediate reinforcement. Both harbor and grey seals were capable of non-imitative vocal learning guided by behavioral feedback: One harbor seal (duration) and one grey seal (f0) moved outside their pre-experimental range, and two harbor seals (f0 & CoG) showed increased vocal flexibility. Future investigations will enable inter-individual comparison and reveal to what extent seals are capable of non-imitative vocal learning.

Poster Session 1 | Poster Wall 44 | Label: PS1.044

Category: Auditory system and acoustic signaling

A Role for Distortions in Drosophila Hearing (?) … a Pilot Study

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As an inevitable by-product of their nonlinear mode of operation, distortion products (DPs) arise in the ears of probably all animals. In humans, they are used as diagnostic tools in clinical audiology, for example to assess the state of auditory health in newborns. DPs are also fascinating phenomenon for psychoacoustic research and artistic endeavours. Despite their indisputable intellectual intrigue, however, DPs only play subordinate roles in our everyday experience and mainstream auditory research.

In stark contrast to these settings, DPs are an essential element in mosquito hearing as they provide mosquito males with the actual sensory cue needed to detect, identify and locate a female within a mating swarm. The female flight tone frequency itself is too high to excite the male's ear, but lower-frequency DPs, generated by the nonlinear mixing of male and female flight tones, lie within their hearing ranges. In fact, our own preliminary research shows that in mosquitoes the sensitivity thresholds for DPs are 10-times lower than those for their primary tone counterparts, highlighting their auditory importance

While DPs have become a key focus in mosquito hearing research, they have remained rather unexplored in other animals, such as e.g. Drosophilid fruit flies, powerful models of (also human) hearing. Insights into a possible role of DPs in Drosophila hearing might help shed light on the utility of DPs across animal ears more widely.

We therefore tested the sensitivity of the Drosophila antennal to DPs generated by simultaneous stimulation with two primary tones. We then compared the corresponding nerve responses, to see (i) whether the fly ears are sensitive to indirect, distortion-generated tones and (ii) if this sensitivity matches (or exceeds) the levels observed for direct, primary-tone mediated stimuli. This research aims to evaluate Drosophila's potential as a model for studying the mechanistic and molecular bases of DPs in animal ears.

Poster Session 1 | Poster Wall 45 | Label: PS1.045

Category: Auditory system and acoustic signaling

Acoustic signals driving species recognition in Xenopus

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Acoustic communication is essential to the reproductive success of many species. Understanding the interplay between the evolution of acoustic signal production and reception is a long-standing challenge.

During courtship, male Xenopus frogs produce advertisement calls to attract female mates. The Xenopus phylogeny consists of ~30 described species, each with a unique advertisement call. Previous work has explored mechanisms underlying the evolution of the vocal central pattern generator. How do acoustic preferences of females evolve alongside the evolution of male courtship calls? Xenopus laevis and X. petersii are two closely related species that diverged ~8.5 million years ago. Each species produces an advertisement call that varies in its spectrotemporal features. Temporally, both species produce calls with a slow and fast rate trill, however the trills of X. petersii are significantly shorter and faster than those of X. laevis. Spectrally, calls of both species consist of a dyad of two dominant frequencies, but the dominant frequencies and the dyad ratio of X. petersii are shifted higher than those of X. laevis.

The spectrotemporal differences between these species creates a natural framework in which to explore evolution of signal production and female preference. An overnight phonotaxis assay in which females are exposed to alternating playbacks of advertisement calls of both species reveals that females are highly responsive to their own species call. Manipulations of spectral and temporal features of natural and synthetic calls will reveal how acoustic preferences of females have evolved.

Identifying the salient signals driving species recognition and vocal preference will provide insights into the neural mechanisms of Xenopus. Results will also provide testable hypotheses for studies aimed at understanding the neuronal circuit properties modified during vocal evolution.

Poster Session 1 | Poster Wall 46 | Label: PS1.046

Category: Auditory system and acoustic signaling

Acoustic behaviour in bottlenose dolphins during two different target discrimination tasks

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Bats and toothed whales use echolocation for orienting themselves and to find prey. We investigated how bottlenose dolphins (Tursiops truncatus) use their dynamic sound sound production and hearing abilities, along with head and body movements, to detect and classify objects. We designed two different experiments with trained, blindfolded dolphins swimming towards and discriminating between two targets. Targets were either balls of three different materials, or hollow cylinders of slightly different wall thickness. Synchronized camera and hydrophone recordings revealed clear differences between individuals in their abilities to identify the correct target. In the ball experiments, dolphin performance was worse than previous experiments with similar paradigm of harbour porpoises, using a different type of echolocation signal. Performance during cylinder experiments were very similar to previous experiments with stationary individuals. Studying how dolphins solved a simplified echolocation task allow a better understanding of the processes behind target detection and discrimination capabilities during natural biosonar circumstances.

Poster Session 1 | Poster Wall 47 | Label: PS1.047

Category: Auditory system and acoustic signaling

Host cricket song pattern recognition across populations of Ormia ochracea

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Unintended receivers (eavesdroppers) often rely on communication signals to detect and locate predators, prey, or hosts. The acoustic parasitoid fly, Ormia ochracea, parasitizes host crickets for the development of their larval young. They find host crickets by recognizing and localizing cricket calling songs. Flies from different geographic regions within the United States are behaviorally specialized to prefer different host cricket species. While the frequency content of cricket calling songs are similar, the temporal patterning of sound pulses are drastically different across cricket species. For example, Floridian O. ochracea prefer the calling songs of Gryllus rubens, which produce long trills with sound pulses and intervals between sound pulses that are 10 milliseconds long, resulting in a pulse rate of 50 pulses/sec. The calling songs of Teleogryllus oceanicus consist of sound pulses that are organized into short and long chirps, each with pulse rates that are different from those of G. rubens. In this study, we elucidate fine-scale temporal pattern preferences from different populations of O. ochracea that prefer different host cricket species. Specifically, we test the hypothesis that flies recognize songs based on different combinations of durations and intervals that result in a specific pulse-rate preference (rather than a preference for a particular duration or interval). We quantify temporal pattern selectivity from flies performing walking phonotaxis on a high-speed treadmill system. Preliminary results suggest that flies from different geographic locations prefer a similar range of pulse rates (40-80 pulses/sec). But Floridian flies are more selective and do not respond to some pulse durations and intervals compared to flies from other populations. These results support the idea that different fly populations differ in their temporal pattern selectivity.

Poster Session 1 | Poster Wall 48 | Label: PS1.048

Category: Electrosensory system

Evolution of Electric Organ Discharge (EOD) in African weakly electric fishes: Genomics and behavioral ecology of a magic trait

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The mormyrids comprise a species-rich group of African weakly-electric fish which has undergone an enormous radiation. This is particularly true for the genus Campylomormyrus which consists of about 15 closely related species mostly restricted to the Congo basin. In combined molecular, electrophysiological, and behavioural studies, we demonstrate that (1) cryptic species are hidden behind morphometrically similar (but electrophysiologically divergent) morphotypes, (2) divergence in Electric Organ Discharge (EOD) is associated with small, but significant morphometric changes regarding the feeding apparatus, and (3) EOD is the trigger of mate recognition. In my talk, I will summarize our work on proximate (genomic, transcriptomic, and histological underpinning) and ultimate (adaptive function for orientation, feeding, and mate choice) determinants of EOD evolution and divergence. I will discuss the idea that the EOD constitutes a “magic trait”, i.e., a variable heritable trait which allows both for ecological diversification and species/mate recognition.

Poster Session 1 | Poster Wall 49 | Label: PS1.049

Category: Electrosensory system

Performance and motor behaviour of Gnathonemus petersii during object detection and size discrimination

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While using active sensory systems animals invest energy to perceive. Amongst these animals, weakly electric Mormyrid fish were found to gradually adapt their sensing strategy to aid this process. We are interested in discovering how Gnathonemus petersii adjust their electromotor strategy to gain sensory information when a task requires the detection and comparison of stimuli.

Twelve fish were trained to swim through a corridor and turn to the correspondent side of the target object. Six of them took part in a size discrimination training and the other 6 in a detection task. In both circumstances, visual input of the objects was blocked by an opaque cloth, therefore the fish very likely solved the task with their electric sense. The discrimination task involved size and relative distance as two congruent cues. In individuals reaching the learning criteria, the distance cue was inverted during test trials. In the animals successfully performing the detection task, the object was shifted farther from the decision area during testing.

Detection performance during the test phase dropped to chance level when objects were farther than 8 cm, which is consistent with the literature. Object size discrimination was achieved either when the relative distance of the two objects was the same or when they differed by no more than one centimetre, suggesting that distance may be a cue in size discrimination.

We observed a noticeable trajectory side bias in some subjects. And such tendency seemed to be more pronounced when the target object was on the correspondent side of the bias. Respectively, during the detection task such bias was reduced in those conditions surrounding the detection threshold. We hypothesized about the role of a side biased trajectory while solving object discrimination and detection tasks. We also quantified the electromotor behaviour throughout the trials to address whether different motor strategies are recruited between learning strategies.

Poster Session 1 | Poster Wall 50 | Label: PS1.050

Category: Electrosensory system

Socializing with fish: an interactive fish robot reveals the principles of communication in groups of weakly electric fish

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During electrocommunication, mormyrid weakly electric fish continuously emit and perceive pulse-type electric organ discharges (EOD). This allows them to exchange identity information based on EOD waveform, as well as contextual information by modifying the inter-discharge intervals (IDI). We previously have shown that shoaling mormyrids frequently echo each other's EOD at specific response times of 14-24 ms and engage in episodes of mutual synchronization. However, it is not fully known what information these signals convey and which social dynamics they evoke. To understand the importance of EOD synchronizations and the rules of social interactions in mormyrids, we paired live fish with an interactive biomimetic fish robot ("ElectroFish"). This allows for full control over the cues we inject into the social system and manipulation of various parameters, such as relative positioning, echo response times, or even deliberate electric jamming. Our fully interactive robot, which operates in closed-loop both electrically and locomotorically, reliably elicits social following behavior and mutual EOD synchronizations with live fish. We analyzed fish-robot interactions and found that synchronizations occur when social partners are in close vicinity. Fish display more following behavior towards an echoing robot when compared to random playback sequences, which indicates that well-directed echoes are important for social cohesion. By replicating and modifying the way these fish interact with each other, we found that during synchronizations, echo delays of fish remain constant, even when being confronted with unnatural response times of the robot. Additionally, certain robot behaviors can evoke aggressive behavior of the fish. Investigating the rules of social behaviors could eventually decipher the meaning of recurring IDI-patterns and help to understand the underlying mechanisms of electrocommunication.

Poster Session 1 | Poster Wall 51 | Label: PS1.051

Category: Electrosensory system

Strategies for context-specific learning in weakly electric fish

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Learning enables animals to adapt to changing environments. Simple forms of learning are known to be widely distributed throughout the animal kingdom, while complex forms of learning, such as context dependent learning, have mainly been studied in higher order vertebrates. Using a two alternative forced-choice paradigm, we investigate whether G. petersii (mormyrids) is capable of context specific object/place association. These fish produce electric signals (EOD) to orient in their environment. They can change their EOD rate depending on demand.

Fish were trained to distinguish between sparse and dense environments. Fish were trained to indicate the perceived context by swimming to either of two spatially fixed goal compartments that contained a plastic or a metal object. Hence, fish could solve the task by either associating a context with these objects or with a fixed location.

Preliminary data shows that fish are capable of context-dependent learning for which fish appear to associate both target object identity and object position with the context. Furthermore, task-acquisition is associated with altered swimming patterns. Fish took stereotypic paths through the set-up that changed when fish learned the task; e.g., one fish initially swam along the border of the setup, entering only one of the two goal compartments. Task-acquisition in this individual was associated with a switch of the trajectory towards sampling the arena from the middle of the setup. In general, inbound trajectories are more stereotyped than outbound trajectories. Similarly, fish sample their environment more (EOD/cm) on the outbound path. Sampling along the stereotyped trajectories appears to be object-specific, indicating that individual objects are of more importance in the evaluation of the context. Indeed, removing single objects increased the likelihood of incorrect choices during probe trials.

Poster Session 1 | Poster Wall 52 | Label: PS1.052

Category: Electrosensory system

Characterising a new class of aerial electroreceptor in bees

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Recently, aerial electroreception has been demonstrated in several arthropod species, as they can perceive weak electric fields. In bees, electrical cues have been shown to play a role in both the communication between bumblebees (Bombus terrestris) and flowers and between individual honeybees (Apis mellifera) during the waggle dance. In all aerial electroreceptive species studied to date, the described mechanism relies on electromechanical actuation of a charged sensor, such as a mechanoreceptive hair or for honeybees, the antennal flagellum via Johnston's organ. Such sensors therefore possess a multimodal function. Here, we propose a new candidate aerial electroreceptor, the placode sensilla, a receptor that is both rich in sensory neurons and abundant on the antennal flagellum. Placodes have been regarded as olfactory receptors in Hymenoptera and other insect orders. We generate the first 3D reconstruction of the ultrastructure of a bumblebee placode and additionally, using atomic and electrostatic force microscopy (AFM, EFM), present a detailed map of the surface topography and electrostatic profile of the placodes of honeybee drones. EFM results reveal that the placode membrane always presents a heterogenous charge distribution, with the pore-rich outer rim holding a higher charge than the central depression and surrounding cuticle. We hypothesise that this electrical contrast both within and between placodes may not only enhance olfaction by attracting odour molecules to the pores, but also enable electroreception by establishing electrical interactions with sources of external charge. The role of these electrostatic properties in sensing electric signals is investigated using electroantennography combined with bespoke electric field and olfactory delivery systems. Together, this multidisciplinary research aims to advance our understanding of the structure and putative electroreceptive function of the placodes, a prominent sensory receptor of the bee antenna.

Poster Session 1 | Poster Wall 53 | Label: PS1.053

Category: Electrosensory system

Population-level spatial coding of conspecific electrosensory signals in the hindbrain of knifefish

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The ability to localize the source of a signal often relies on complex neural dynamics to extract the spatial information. In weakly electric fish, behavioral data suggests that detection and localization of conspecifics is extremely sensitive. Little is known about the neural coding principles involved in encoding the spatial information of conspecific signals beyond the fact that the lower electrosensory areas are organized in topographic maps. Here we use a combination of neural recording and large-scale modelling to examine the encoding pattern and accuracy of the population of primary sensory neurons. Our data shows that a specific subpopulation of neurons encode the spatial information significantly better suggesting that the spatial processing pathway starts diverging early in the sensory system. Furthermore, we show how network inputs shape the information content and enhances spatial contrast.

Poster Session 1 | Poster Wall 54 | Label: PS1.054

Category: Electrosensory system

Specialized signals for conspecific electrolocation in weakly electric fish

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Brown ghosts have been widely used as a model to study social communication in fish. During social encounters, the sinusoidal electric fields of these South American gymnotiform fish are heavily modulated in frequency. This phenomenon (so-called "chirping") has been typically considered to represent some kind of electric code to transmit information related to status, territorial ownership, and eagerness to fight or mate. Our recent work has challenged this view and showed that chirp production relates more consistently with environmental clutter and locomotion rather than other, social-related variables. These findings suggested that electric field modulations could be used for electrolocation purposes.

In this study, we aimed at verifying this hypothesis by assessing the reciprocal positioning of brown ghosts during chirping. The idea is that if chirps are used as probes, they must be used when fish are well positioned to probe the spatial parameters of conspecific electric fields. Recordings of chirps from interacting fish pairs were used to extract body coordinates during chirps and estimate both inter-fish distances and orientations. Moving playback sources were then used to measure more precisely the allocentric and egocentric referencing of chirps. We found that chirps are consistently produced when the brown ghost foveal RF is oriented towards the playback mimics and at ranges within which rostro-caudal asymmetries in sinusoidal electric fields can be discriminated. Eventually, by simulating electroreceptor responses and electric images induced by chirps during social encounters, we provide further evidence to corroborate the idea that chirps can be used as probes for conspecific electrolocation.

Poster Session 1 | Poster Wall 55 | Label: PS1.055

Category: Electrosensory system

Electrocommunication signals of the brown ghost knifefish might indicate submission during competitions – deep learning applied to animal behavior

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Communication is vital in resource competition among animals, enabling the establishment of dominance hierarchies and minimizing the need for physical confrontations. In electric fish the role of chirps, a common type of electrocommunication signals, in competitions has been discussed controversially. Previous experiments were restricted to physically separated fish, because detection of chirps in freely interacting fish is impossible with conventional techniques. This study employs deep learning for automated chirp detection in freely moving Apteronotus leptorhynchus, aiming to clarify the role of chirps in staged competition experiments.

We used a deep convolutional neural network for object detection and fine-tuned it on labeled spectrograms of chirping fish. Post-processing routines and custom algorithms associate detected chirps with the specific fish emitting them. This enabled detection and attribution of chirps to individuals, resulting in a substantial ataset of approximately 50,000 chirps from freely interacting fish.

Our first application focuses on chirping patterns and their behavioral correlations: We found a significant correlation between chirps emitted by subordinate individuals and the cessation of agonistic interactions during competitions for shelter. This suggests that chirps may serve as submissive signals, influencing the dominant fish to cease attacks during ritualized fights.

Beyond controlled conditions, our automated approach is applicable to field recordings: Previous work demonstrated the possibilities of markerless tracking of many individuals in their natural habitat by recording their generated electric fields on electrode grids. We build upon that by adding the possibility to extract the full electrocommunication repertoire from such recordings. For the first time, this provides all the tools for extracting movements and communication signals for quantitative ethology in undisturbed populations of electric fish.

Poster Session 1 | Poster Wall 56 | Label: PS1.056

Category: Electrosensory system

Scaling rules in a cerebellum-like circuit in a weakly electric mormyrid fish

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Brain size and neuron counts are often used in comparative studies to approximate processing capacity and draw conclusions about evolutionary strategies across species. However, these metrics can also be used within one species during growth to understand how circuits adapt to larger bodies and meet changing sensory processing demands. The weakly electric mormyrid fish Gnathonemus petersii can continue to grow throughout adulthood, and as it grows its brain mass may increase more than 10-fold. It has been shown in closely-related species that large numbers of granule cells, the most abundant neuron type, are added to the cerebellum and eminentia granularis posterior (EGP) in adulthood, but how this growth scales in relation to other structures and cell types that are part of the same processing pathways is not known. Granule cells in the EGP are used to generate predictions of sensory input that are then integrated in the electrosensory lobe (ELL) with sensory information coming from electroreceptor afferents. We used unbiased stereology to quantify densities of electroreceptors and the major neuron types of the ELL to investigate how the ELL balances this large increase in central input from EGP with peripheral inputs. Consistent with prior studies we found evidence from cell counting and labeling of adult born cells that the number of granule cells increases isometrically with brain mass, as does the volume of the molecular and cell layers of the ELL. Interestingly, both output cell types of the ELL markedly decrease in density as the animal grows, which matches a corresponding decrease in density in the electroreceptors on the skin, while Purkinje-like interneurons (the main recipient of granule cell input), maintain a constant density. These results suggest that increased convergence of Purkinje-like cells onto output cells may compensate for greater distances between electroreceptors and therefore scarcer sensory information relative to body size.

Poster Session 1 | Poster Wall 57 | Label: PS1.057

Category: Electrosensory system

Burst firing generates invariant coding of natural electrocommunication stimuli

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Understanding how neurons process sensory information for successful environmental interaction remains a central problem in neuroscience. In particular, animals need to recognize various representations of sensory inputs from the same "object" under different conditions. This is thought to be achieved in the brain by having neurons respond in a similar manner (i.e., invariantly) to sensory input through identity-preserving transformations. While such invariant representations have been observed across systems and species, the mechanisms by which they are achieved in the brain remains poorly understood to this day.

In our study, we explored how burst firing contributes to the invariant representation of electrocommunication signals ("chirps") in the electrosensory system of weakly electric fish. We used Neuropixels probes for multi-unit recordings from ELL pyramidal cells. We found that, at the population level, bursts of action potentials tended to be elicited more reliably and similarly by different stimulus waveforms, leading to a more invariant representation than that obtained by considering the entire spiking activity. Interestingly, isolated spikes provided the least invariant representation. Additionally, we developed a biophysical model of ELL pyramidal cells to explore the intrinsic mechanisms leading to such invariant findings, highlighting the crucial role of somato-dendritic interactions in generating invariant representations. Finally, we assessed how downstream torus neurons in the hierarchy of the electrosensory circuit decode these invariant representations by developing and training a deep neural network (DNN) optimized for invariance. Overall, our results show a novel function for burst firing in establishing invariant representations by ELL pyramidal cell populations of natural electrosensory communication stimuli and suggest that such representations are decoded by downstream TS neurons to further optimize invariance.

Poster Session 1 | Poster Wall 58 | Label: PS1.058

Category: Electrosensory system

The effect of urethane and MS-222 anesthesia on the electric organ discharge of the weakly electric fish Apteronotus leptorhynchus

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Urethane and MS-222 are agents widely employed for general anesthesia, yet, besides inducing a state of unconsciousness, little is known about their neurophysiological effects. To investigate these effects, we developed an in vivo assay using the electric organ discharge (EOD) of the weakly electric fish Apteronotus leptorhynchus as a proxy for the neural output of the pacemaker nucleus. The oscillatory neural activity of this brainstem nucleus drives the fish's EOD in a one-to-one fashion. Anesthesia induced by urethane or MS-222 resulted in pronounced decreases of the EOD frequency, which lasted for up to 3 hours. In addition, each of the two agents caused a manifold increase in the generation of transient modulations of the EOD known as chirps. The reduction in EOD frequency can be explained by the modulatory effect of urethane on neurotransmission, and by the blocking of voltage-gated sodium channels by MS-222, both within the circuitry controlling the neural oscillations of the pacemaker nucleus. The present study demonstrates a marked effect of urethane and MS-222 on neural activity within the central nervous system and on the associated animal's behavior. This calls for caution when conducting neurophysiological experiments under general anesthesia and interpreting their results.

Poster Session 1 | Poster Wall 59 | Label: PS1.059

Category: Electrosensory system

Extracellular potassium concentration is a major determinant of the firing frequency in the pacemaker nucleus of a high-frequency electric fish, Apteronotus leptorhynchus

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The high frequency electric organ discharges of the weakly electric brown ghost knifefish (Apteronotus leptorhynchus) originate from the medullary unpaired nucleus, the pacemaker nucleus, in which neurons are coupled each other and synchronously fire 1:1 with the electric organ discharges at unusually high frequencies (600-1100 Hz). The discharge frequency is individually fixed and constant over hours and days. However, A. leptorhynchus exhibits a pronounced sexual dimorphism in its discharge frequencies — males discharge at higher frequencies than females, with little overlap between the sexes. While little is known about ionic mechanisms for frequency control intrinsic to the pacemaker nucleus, previous studies have discovered sex-specific differences in the morphology of the astrocytic syncytium that envelopes the pacemaking neural network and may regulate extracellular potassium concentration by their potassium buffering mechanism. This prompted us to examine the relationship between extracellular potassium concentration and firing frequency of the pacemaker nucleus. The pacemaker nucleus was isolated in a submerge-type in vitro recording chamber while extracellular potentials of the synchronized neural activity were monitored. The nucleus was exposed to physiological perfusate with various concentrations of K+ with osmotic pressure compensated with choline chloride. While reducing postassium ion concentrattion to 0 mM from the baseline condition (3.5 mM) decreased firing frequency by ~30 Hz, increasing postassium ion concentrattion to 16.5 mM increased the frequency by ~50 Hz. The positive relation appears to be due to increased potassium equilibrium potential and thus excitability of the pacemaking neurons. The abundance of astrocytic syncytium in females is suggested to reduce female discharge frequencies by its potassium buffering capacity.

Poster Session 1 | Poster Wall 60 | Label: PS1.060

Category: Evolution and development

The Crown Jewel of Stress Protection? Investigating the Role of the Major Royal Jelly Proteins in Stress Protection

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In the Western honey bee (Apis mellifera) the most highly expressed genes in the brain are the Major Royal Jelly Proteins (MRJPs). These nine recently duplicated genes have a well-known nutritive role, however the functions of these genes within the brain are still poorly understood. Previously, we have developed lines of MRJP-expressing Drosophila melanogaster to further investigate the role of these genes in the brain. Our transcriptomic data showed expression of these genes results in a nurse-like brain state and influence in stress reduction pathways within the brain tissue. To further investigate the potential protective nature of the MRJPs, we utilized both transgenic Drosophila lines and dsRNA knockdowns in honey bees. We exposed both honey bees and flies to the neurodegenerative herbicide Paraquat before putting them through a sucrose discrimination assay. Since Paraquat is also known to cause oxidative stress, we further tested brain tissue for markers of oxidative stress. Our results help elucidate the roles the MRJPs play in the honey bee brain in their transition from nursing to foraging.

Poster Session 1 | Poster Wall 61 | Label: PS1.061

Category: Evolution and development

Toward understanding the molecular basis of behavioral evolution in Hymenoptera: insight from molecular developmental analysis of mushroom bodies in the honey bee brain

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Hymenoptera includes species exhibiting a wide variety of behavioral traits, which makes this insect order useful for investigating the brain basis of behavioral evolution. In the European honey bee, mushroom bodies (MBs), a higher-order center of the insect brain, comprise three class Kenyon cell (KC) subtypes: lKCs, mKCs, and sKCs. It is suggested that the increase in the number of KC subtypes is related to the behavioral evolution in Hymenoptera. To elucidate the molecular basis underlying the increase in the number of KC subtypes, we conceived an interspecific comparison of the molecular basis of the KC subtype development. Here, we analyzed the development of KC subtypes in the honey bee. We first identified the pupal stage at which each KC subtype is produced from the MB neuroblasts (NBs) by injecting EdU, which labels proliferating cells, into the heads of pupae at various pupal stages and examining the KC subtypes labeled with EdU after the emergence. The results indicated that the lKCs, mKCs, and sKCs are produced until 30 hours after pupation (hap), between 30 and 50 hap, and between 50 and 120 hap, respectively. To identify candidate genes involved in the production/differentiation of each KC subtype from NBs, we performed an RNA-seq analysis using the proliferating MB cells isolated at the pupal stages when each KC subtype is produced, and identified genes upregulated at each pupal stage. We also performed in situ hybridization of these candidate genes using the brains of pupae at the stage when each gene is upregulated. Some genes are preferentially expressed in the center of the pupal MBs where MB NBs are located. These results suggest that pupal MB NBs differentiate into each KC subtype by changing their gene expression profiles in a pupal stage-dependent manner. Based on these results and previous studies, we propose a model of the molecular mechanisms of MB development that underlies the increase in the number of KC subtypes in Hymenoptera.

Poster Session 1 | Poster Wall 62 | Label: PS1.062

Category: Evolution and development

Modification of motor cortical circuitry underlies the evolution of behavioral novelty in the singing mouse

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To understand how neural circuits evolve, our strategy is to identify neural circuit modifications among closely-related species with large behavioral divergences. Two rodents with divergent vocalization behaviors are Mus musculus (lab mice) and Scotinomys teguina (singing mice). While most rodents produce short, ultrasonic vocalizations (USVs), singing mice display a novel vocalization of songs that are human-audible, stereotyped, and seconds long. Manipulation and recording experiments have indicated a role for orofacial motor cortex (OMC) in this behavior. Here, we investigate the following OMC circuit modifications that could result in the evolution of novel behavior: (1) novel projections, (2) increased neural arborization, or (3) increased number of neurons that project to downstream targets.

To test for novel projections, we identified bulk projection targets of OMC neurons using viral tracing and whole brain imaging. In both species, OMC neurons project to identical regions including the contralateral cortex, striatum, thalamus, periaqueductal grey (PAG), and others (3 mice per species). We next wanted to determine whether there were differences in projection strength at single-cell resolution by using MAPseq, a high-throughput RNA barcoding technique. We found no difference between species in the number of projections individual neurons had to each target region. But in the singing mice, a larger proportion of OMC neurons project to the PAG and a temporal cortical area (singing mice: 5114 neurons, 7 animals; lab mice: 71704 neurons, 5 animals). We also found that the increased projection to temporal cortex and PAG from OMC was driven by neurons with few collaterals.

In summary, we found evidence for expansion of existing vocal motor circuits in the singing mice, which may explain their species-typical vocal behaviors. Our work demonstrates how comparing related species can give insight into the neural mechanisms of behavior and neural circuit evolution.

Poster Session 1 | Poster Wall 63 | Label: PS1.063

Category: Evolution and development

Ostracod neuroanatomy reveals ancient origin of mushroom bodies and central complex in Pancrustacea

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Learning is an adaptive behavior allowing animals to adjust to environmental conditions and supporting many essential skills such as food caching and successful navigation to a home burrow. In insects, the major brain structure associated with learning and memory is called the mushroom body, a multisensory integration center that has a well-established role in learning. More recent work has uncovered insect-like mushroom bodies in some crustacean lineages such as stomatopods, raising questions about whether these structures have evolved independently or were inherited from a common ancestor. Ostracods, commonly known as seed shrimp or sea fireflies, present a unique opportunity to investigate the evolutionary origins of this learning and memory center, as well as other structures potentially conserved across Pancrustacea. Ostracods are the most speciose members of Oligostraca, a clade sister to all other pancrustaceans and the oldest known crustacean group in the fossil record. Despite their phylogenetic significance, knowledge of ostracod neurobiology is extremely limited, with little known about the adult brain. Investigating ostracod neuroanatomy is essential to unraveling questions about pancrustacean brain evolution. In this study, we used synchrotron micro-tomography, 3D reconstruction and immunohistochemistry to examine the brain morphology of two cypridinid ostracod species, Vargula tsujii and Skoksbergia lerneri. Here, we present the first model of an adult ostracod brain. Staining with a known mushroom body marker, anti-DC0, provides evidence for the existence of mushroom bodies within this taxa. We have also characterized ostracod eye morphology and described a central complex similar to those found in insects. This research expands our limited knowledge of ostracod neuroanatomy, allowing a more accurate reconstruction of the ancestral pancrustacean brain and the evolutionary origin of learning and memory centers.

Poster Session 1 | Poster Wall 64 | Label: PS1.064

Category: Evolution and development

The co-evolution of male song and female song preference in cricket

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Speciation is driven and maintained by the co-evolution of male and female mating behaviors. While mate-attracting signals such as mating songs are often relatively straightforward to examine, the associated signal preferences are difficult to estimate as they require extensive behavioral experiments or neural data. Crickets have been a model system for mate recognition and behavioral evolution due to their simple but highly diverse songs and fast speciation times. Male crickets produce species-specific songs consisting of pulses organized in two timescales. Female crickets respond positively to the song by phonotaxis. While individual species have unveiled the evolution of the cricket song and auditory processing mechanisms, a holistic overview of behavioral diversity in the taxon is missing. Here, we present behavioral data of nineteen cricket species consisting of male song properties and female song preferences. Species-specific songs in the short timescale can successfully attract conspecific females, suggesting that the song and preference on the short timescale support and have co-evolved for species recognition. By contrast, the song properties in the long timescale are partially mismatched to the female preference, indicating divergent selection pressures acting on both timescales. Song and song preference on both timescales correlate only weakly with phylogeny, indicating the high evolvability of the system. Interestingly, male songs of a species bred in captivity deviate from female preference, illustrating behavioral divergence in the absence of selection pressure. Our results highlight the behavioral diversity and co-evolution of song and song recognition within the cricket taxon and provide a foundation for further studies on the evolution of the neural circuits underlying song production and song recognition.

Poster Session 1 | Poster Wall 65 | Label: PS1.065

Category: Evolution and development

High vocal flexibility impedes the evolutionary adaptation of bat echolocation signals

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Phenotypic plasticity is a ubiquitous solution used by organisms to cope with variable environments and plays an important role in driving phenotype evolution. However, little is known about how phenotypic flexibility, which is the reversible form of phenotypic plasticity exhibited by the same individual, contributes to phenotype evolution. Here, leveraging the rich knowledge of bat echolocation behavior, we experimentally quantified the flexibility, estimated the functional significance, and examined the evolutionary consequences for two acoustic phenotypes: call frequency and call amplitude of bat echolocation signals. We found that call amplitude was 16 times mechanically and 18 times functionally more flexible than call frequency. Surprisingly, echolocating bats showed evolutionary adaption of call frequency, but not call amplitude, to their main foraging habitats. A simple fitness model was able to explain this paradoxical relationship between phenotypic flexibility and evolutionary adaptation. Thus, evolution favors phenotypes of moderate, but not high flexibility.

Poster Session 1 | Poster Wall 66 | Label: PS1.066

Category: Evolution and development

Males in more complex social groups have larger brains and greater social competence: evidence from wild cichlid fish in Lake Tanganyika

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The social brain hypothesis (SBH) argues that the cognitive demands of living in complex social groups select for increased investment into the brain. Although controversial in both interpretation and empirical support, the SBH remains a topic of vigorous debate in neuroethology. Here we investigate the relationship between social complexity and relative brain size in wild cichlid fish, Neolamprologus multifasciatus in a natural context where common ecological confounds are absent, and only social organization differs among individuals. Using a combination of automated underwater video analysis, behavioral scoring, and neuroanatomical measurements, we find a significant positive correlation between social complexity experienced by individuals and their relative brain size, with the most pronounced effects observed in the dominant males in each group. To test whether this effect is due to incidental developmental advantages of living in large groups, or alternatively whether large-brained males have some social advantage, we allowed size-matched males with differing relative brain sizes to compete for access to groups of females. We found that males with larger brains were more likely integrate into larger groups of females than were smaller-brained males. We then tested whether this social advantage was due to increased male-male competitive ability, finding that larger-brained males were not more likely to win in territorial conflicts with smaller-brained males. Combined, these results suggest that large-brained males have greater social competence (in the form of male-female interactions), but not greater competitive abilities (male-male interactions). Overall, our results demonstrate unexpected support for the SBH in a wild fish species under natural contexts, and go further to demonstrate a direct social advantage of larger-brained males.

Poster Session 1 | Poster Wall 67 | Label: PS1.067

Category: Evolution and development

An unusual birthing behavior protects the progeny of the arsenic-resistant nematode Tokorhabditis tufae

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Due to anthropogenic change, many environments will become more extreme. Which animals can adapt to these changes, and through which mechanisms, is poorly understood. We can gain insights from organisms that naturally thrive in extreme habitats to learn how animals can become resilient to harsh environments. In this pursuit, we are studying Tokorhabditis tufae, a novel nematode species we recently isolated from Mono Lake, an arsenic-rich lake incompatible with most animal life (Shih et al., 2019). We found that T. tufae adults are resistant to arsenic, but early-stage progeny are sensitive. T. tufae demonstrates a rare birthing behavior that promotes the survival of its progeny in arsenic. While almost all nematodes reproduce by laying eggs, T. tufae hatches its progeny internally, providing nutrients (Yamashita et al., 2023) and protecting them from toxins until they reach a stress-resistant larval stage. Pharmacology and immunostaining reveal that birthing in T. tufae is under distinct neurobiological control compared to egg-laying in species like C. elegans, with changes that likely support the retention of eggs and subsequent internal hatching. Gestating T. tufae progeny also demonstrate behaviors that may support live birthing. For example, progeny in the uterus are behaviorally quiescent during development but transition into a mobile state correlated with the development of stress-resistant traits. We hypothesize that this mobility facilitates birthing of well-developed larvae through their own movement. Thus, T. tufae demonstrates adaptations to its arsenic environment that involve behavioral, developmental, and physiological alterations from the ancestral egg-laying state of nematodes. Understanding the mechanisms of these changes may offer insights into how neural circuits can evolve to generate new adaptive behaviors, and how animals can survive environments polluted by toxins like arsenic, a significant and worsening issue with anthropogenic change.

Poster Session 1 | Poster Wall 68 | Label: PS1.068

Category: Evolution and development

Early postnatal development of vocal flexibility in the echolocating bat

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Most bats strongly rely on echolocation for navigation and foraging. Probably due to the need to cope with dynamic environments, all echolocating bats possess the capacity to flexibly adjust the structure of their biosonar calls, i.e., vocal flexibility. However, little is known about when and how newborn bats acquire such vocal flexibility during postnatal development, although many previous studies have investigated the postnatal development of several phenotypes of bats, such as the body size, echolocation call, and flight behavior. Here, we investigated the postnatal development of vocal flexibility in the infant greater horseshoe bat, Rhinolophus ferrumequinum, using an online auditory feedback perturbation paradigm. Our results revealed that the isolation calls of the infant bat contained multiple harmonics, and both the fundamental and peak frequencies of the call increased with age, while the number of harmonics decreases. The ability of the infant bat to compensate for the frequency perturbation of emittedcalls was evident already two weeks after birth, and the magnitude of compensation improved with age, reaching a level similar to that of adult bats at around two months of age. This research showed that the spectral vocal flexibility of bats emerged much earlier than the adult echolocation calls and powered flight capability.

Poster Session 1 | Poster Wall 69 | Label: PS1.069

Category: Evolution and development

Mechanisms of Drosophila escape circuit evolution

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The acquisition of novel traits during species diversification enables animals to optimize their fitness in new ecological niches. Predation risk, which poses an imminent threat to survival and reproductive success, is a strong selective force that shapes prey behavioral repertoires and their underlying neural circuits during evolution. Depending on the expansion speed of an artificial looming stimulus, Drosophila melanogaster selects between executing a faster short mode escape sequence or a slower but more stable long mode takeoff. The switch to shorter-duration escapes depends on Giant Fiber (GF) interneuron activation. We have previously shown that D. melanogaster GFs confer a survival advantage under naturalistic predation conditions (Chai et al. in prep). In the wild, drosophilids in different habitats are challenged by different constellations of predators that employ distinct attack strategies. Although large axon-diameter giant neurons are specialized for speedy escapes, they also drive uncontrolled responses, which might not always be advantageous. Here we compared the visually-evoked escape responses of D. melanogaster with its close relatives, D. simulans and D. mauritiana. Our results indicate that, when challenged with moderately fast expansion speeds, D. simulans and D. mauritiana executed significantly more short mode takeoffs compared to D. melanogaster. We hypothesize that species-specific adaptations in GF physiology and/or synaptic connectivity underlies escape mode selection variation. We are now developing transgenic tools for cell-type-specific targeting to determine the neural circuit basis of escape mode selection in non-model Drosophila species. By applying an evolutionary comparative framework in the context of this behavioral neuroscience paradigm, we aim to identify the neural elements that undergo the greatest selection pressures giving rise to behavioral diversity during speciation.

Poster Session 1 | Poster Wall 70 | Label: PS1.070

Category: Evolution and development

Locomotor maturation during early development in a small vertebrate

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Most animals are born with a minimal repertoire of behaviors, which is gradually expanded as they grow and adapt to their changing needs, environments, and body morphology. The emergence of new functional capacities is associated with changes in the brain architecture, as new neurons become functional and new connections are formed. How brain maturation orchestrates phenotypic transformations during development is still an open question in behavioral neuroscience. Here, we investigate this question studying locomotion in Danionella cerebrum, a novel vertebrate model whose brain remains small and transparent up to the adult stage, thus offering a unique opportunity to perform large-scale monitoring of brain activity with cellular resolution through its entire lifespan. Our interest for locomotion is twofold. Firstly, Danionella displays a phenotypic transition from long continuous swimming at the larvae stage to intermittent burst-and-glide swimming in juveniles. Furthermore, locomotion engages extended circuits and requires temporal and spatial coordination of the brain activity; it is thus particularly well adapted to study brain-wide circuit maturation.

A customized freely-swimming assay was developed to obtain a comprehensive dataset on exploratory behavior throughout development. This setup enables the examination of swimming kinematics with high spatial resolution and across various time scales. On the short timescale of seconds, hydrodynamic arguments together with computational fluid dynamics simulations are employed to establish a connection between phenotypic changes and morphologic growth. On a longer timescale, spanning tens of minutes, a Markovian-based state space model is utilized to uncover the inherent structure of exploration; we defined a postural space to display the dynamic of the so defined behavioral units. Finally, calcium imaging across development will elucidate the functional role of neuronal circuits underlying this phenotypic transition.

Poster Session 1 | Poster Wall 71 | Label: PS1.071

Category: Evolution and development

Is the Drosophila DRA an evolutionary novelty of higher diptera shaped by molecular changes in homothorax locus?

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Most free-living insects rely on a combination of various visual stimuli for navigation, a famous example being the pattern of polarized skylight. All insect eyes consist of repetitive functional units called ommatidia, whose photoreceptors define specific ommatidial subtypes, being tuned to different kinds of visual modalities, like brightness, chromatic content, or the angle of polarization. Despite the great morphological diversity of insect eyes, the distribution of ommatidial subtypes (together forming a retinal mosaic for color and skylight polarization vision) shows remarkable similarities across species. In Drosophila, the homeodomain transcription factor Homothorax (hth) is both necessary and sufficient for specifying polarization-sensitive ommatidia in the 'dorsal rim area' (DRA) of the adult eye. Functionally analogous DRA ommatidia for detecting polarized skylight are found at the dorsal margin of most insect eyes analyzed to date. Although the hth gene is conserved across species, it is currently unknown whether its role in retinal patterning is also conserved. To reveal the mechanisms shaping the circuits processing skylight polarization downstream of the retinal mosaic across insects, we followed a multi-disciplinary approach: First, we identified evolutionarily conserved regulatory sequences within the hth locus that drive expression in the DRA of flies. Secondly, we generated antibodies against Hth of honey bees, butterflies, mosquitos and used them to compare pupal retinal expression across these species. Surprisingly, we discovered that the Hth-dependent mechanism for specifying DRA ommatidia appears to be an innovation of higher flies. Finally, we used scRNAseq to reveal the transcriptomes of the cellular units processing polarized skylight in flies, to identify similar cell types in other species. Taken together, our work establishes the foundation for studying the evolution of skylight polarization circuitry across species.

Poster Session 1 | Poster Wall 72 | Label: PS1.072

Category: Evolution and development

Cell types and Circuits Underlying Cuttlefish Camouflage

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Many cephalopods camouflage, evading predators and surprising prey by matching their appearance to their surroundings. This behavior requires animals to observe their environment, process texture information, and reproduce a matching texture on their skin. Not only is this behavior a remarkable feat of visual perception, but the lineages giving rise to cephalopods and vertebrates diverged very early during animal evolution, providing a fascinating opportunity to study the convergent evolution of sensory computations. While the brain areas involved in camouflage have been coarsely mapped, the cell types and circuits underlying the behavior remain largely unknown.

To understand the genetic basis of camouflage in the European cuttlefish *Sepia officinalis*, we are assembling the genome of an adult cuttlefish using various sequencing techniques. Using the genome as a reference, we profile cell type diversity in the cuttlefish brain using single-nucleus RNA sequencing. Further, we visualize the cell types by mapping the spatial expression of marker genes using *in situ* hybridization and spatial sequencing methods to generate a comprehensive molecular map of the cuttlefish brain. Ultimately, we aim to inform physiological studies of the identified cell types to link their molecular profiles to their role in camouflage behavior.

Poster Session 1 | Poster Wall 73 | Label: PS1.073

Category: Evolution and development

The development of synaptic partner choice and a robust representation of skylight polarization in the fly optic lobes

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The process of neuronal partner choice and neuronal circuit assembly is a highly complex process, in which closely related neurons exhibit highly specific divergent connectivity in the adult brain. To achieve this final pattern, several mechanisms occur in a manner controlled both in time and space, throughout development. We are interested in the molecular and cellular mechanisms underlying the formation of neural circuits that process specific visual cues for navigation in the fruit fly. Using the synaptic pathway for processing skylight polarization as a model system, we investigate the downstream targets of photoreceptors in the dorsal rim area (DRA) in the developing visual system of Drosophila. Photoreceptors in the DRA manifest a modality-specific pattern of synaptic connectivity as they avoid contact with postsynaptic targets of their neighboring non-DRA (i.e. color sensitive) photoreceptors.

We aim to compare the selective targeting of DRA.R7 and DRA.R8 photoreceptor axons towards their respective downstream postsynaptic partners, distal medulla neurons, known as Dm-DRA1 and Dm-DRA2, by studying their developmental dynamics. We hypothesize that the robust representation of navigational information processed by DRA neurons relies on spatio-temporal separation of partner choice and that their probabilistic filopodial exploration contributes to a cell type-specific reprogramming of development, resulting in a modality-specific modulation of synaptogenic encounters.

To test our hypothesis, we use live imaging of ex-vivo brain cultures in combination with two photon microscopy and connectomic analysis to study both cellular encounters and the filopodial dynamics of pre- and postsynaptic membranes. In addition to testing this in wild type flies, specific perturbation experiments will be performed through manipulating temperature and genetic reprogramming of DRA photoreceptors.

Poster Session 1 | Poster Wall 74 | Label: PS1.074

Category: Evolution and development

The Development of the Representation of Skylight Polarization in the Central Brain of Drosophila

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A central challenge in neuroscience is to understand how the brain achieves precise connectivity despite many neurons developing in close proximity. Our research focuses on elucidating the mechanisms underlying modality-specific wiring in the Drosophila visual system, particularly in those circuits processing of skylight polarization downstream of the dorsal rim area (DRA). We use photoreceptors and MeTu neurons as model system. MeTu neurons connect the optic lobes with the central brain which provides insights into the establishment of a robust topographic representation of skylight information.

The ability of neurons to promiscuously form synapses with other neurons and the development of spatial-temporal specifcity during synaptogenesis create an apparent paradox. We hypothesized that the DRA circuitry uses spatiotemporal separation of synaptogenic interactions to regulate specific encounters, ensuring modality-specific wiring. Furthermore, we suggest that the morphological variability of overlapping target cells contributes to a non-deterministic distribution of synapses, which promotes robust circuit function at the population level.

To test our hypothesis, we follow two approaches: First, we quantitatively investigate filopodial dynamics of pre- and postsynaptic cells by utilizing multi-photon ex vivo imaging in both wild-type and brains with targeted manipulations. Secondly, we investigate the variability in neuronal morphology and synaptic distribution using EM-based connectomics. We aim to unravel the mechanisms underlying the neuronal development of robust wiring for computing skylight polarization. MeTu neurons connect the optic lobes with the central brain which provides insights into the establishment of a robust topographic representation of skylight information.

In conclusion, by investigating the intricate balance between precision and plasticity in synaptic wiring, our findings will provide insights into neural circuit development and function.

Poster Session 1 | Poster Wall 75 | Label: PS1.075

Category: Evolution and development

Context-dependent patterning of Drosophila courtship song mirrors phylogenetic relationships

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Sexual reproduction relies on the production and perception of species specific communication signals. Such signals provide key information on the sex, species, quality, and state of the potential mate. Courtship displays are diverse and dynamic to communicate this information, signaling desirability and attractiveness. A courter has to be flexible and accurate to succeed. We study the Drosophila's acoustic signaling during courtship, a dynamic and context-dependent behavior. Males sing a species-specific song to woo females, where females are more likely to mate with males singing a conspecific song. The characteristics, and notably, the behavioral contexts in which these acoustic signals are produced differ by species. To court successfully, males have to produce the species-specific signals at the right time and context. To understand how the behavioral contexts shaping production of song event and type evolve, we use computational modeling of data from audiovisual recordings of closely related D. santomea and D. yakuba, more distantly related D. teissieri, and most distant D. melanogaster. Context-dependent singing clusters by phylogeny, with correlations between the number and effect of shared predictive behavioral features, and the evolutionary closeness between species. Predictive features are more likely shared between closely related species, cross-validation between these models score well, but not between distantly related ones. Similarly, the temporal dynamics of the features preceding the song event, or filters, mirror phylogeny: filters that predict the same song type are shaped more similarly in closely related species. Short-timescale differences in filter shapes and in song type predicted even between closely related species suggest species idiosyncrasies in sensorimotor processing. The courtship song and contexts patterning singing are unique to the species, but their divergence follows a sequence that reflects their phylogenetic relationship.

Poster Session 1 | Poster Wall 76 | Label: PS1.076

Category: Evolution and development

A trade-off between sensory and processing capabilities in the midwater

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The ocean's midwater is a difficult place to live. Sensing their environment, finding food, mates and avoiding predators in this vast volume of water is challenging given the truly three-dimensional nature of the habitat, the viscosity of the water, and limited light. Midwater animals that depend on vision often have disproportionately large eyes. The holopelagic suborder Hyperiidea is an ideal taxon to examine the cost of large eyes because across the 80 genera they show a full range of eye sizes. We ask the question whether there are neural trade-offs when eyes are enlarged. We compare body, eye, and brain size with detailed measures of sensory versus central processing brain regions. Broadly, we find that hyperiids have a significantly smaller brain to body ratio than most animals and that they exhibit far more diversity in optic lobe structure than any other known crustaceans. We also find that eyes, but not brains, scale with body size and that sensory neuropils scale with sensor size but not brain or body size. We found a trade-off between different sensory modes – genera with large eyes, and consequently large optic lobes, have small olfactory lobes. However, the central brain neuropils (central body in this study) do not scale with eye or body size, only with brain size. Finally, we find that the retinotopic optic neuropils scale with eye size while "processing" optic neuropils do not. Together, these findings suggest that some hyperiid amphipods expanded their sensory abilities at the cost of processing abilities. The relatively featureless midwater may not be as cognitively challenging as more structurally complex environments such as coral reefs, but the midwater is extremely challenging regarding sensing the environment. Future work focuses on identifying what processing they are capable of given their reduced brain size and the frequent absence of the lobula and/or lobula plate, typically responsible for higher-order visual processing in other species.

Poster Session 1 | Poster Wall 77 | Label: PS1.077

Category: Evolution and development

Danionella dracula telencephalon is a mosaic of larval, juvenile and adult characters

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The diversity of vertebrate behaviors is reflected in the variability of brain structures, particularly in the telencephalon where the complexity of neural circuitry is assumed to scale with cognitive capacity. Miniaturized animals challenge this assumption. Danionella, a genus of paedomorphic, developmentally truncated minnows closely related to zebrafish, are among the smallest living vertebrates. Like larval zebrafish, they are transparent and lack a fully ossified skull, contributing to growing interest for neurobiology because neural activity across the entire brain can be examined in vivo. Adult Danionella exhibit social and cognitive behaviors typical of adult fishes in a larval-like body. Hence, we asked if the telencephalon appears developmentally truncated. Comparing D. dracula adults to larval, juvenile, and adult zebrafish, we find dramatically reduced cell migration from ventricular layers similar to a larval condition, preventing easy identification of many classically defined teleost brain nuclei. This is most pronounced within caudal pallium subdivisions, giving them a compartmental, lobe-like appearance. Nonetheless, comparing expression of calcium-binding proteins, neuromodulators, transcription factors and olfactory-bulb projections reveals D. dracula possess all major subdivisions of adult teleost telencephalon. However, the caudal pallium's topological organization more closely resembles larval and juvenile zebrafish. We find a similar organizational pattern in 2 other Danionella species. The retention in adult Danionella of larval and juvenile traits in regions that have been functionally compared to isocortex, amygdala and hippocampus in tetrapods raises several questions. Are there behavioral and cognitive tradeoffs for these miniature brains? Or have Danionella evolved novel neuroanatomical and computational strategies for achieving adult-like social and cognitive behaviors with a larval-like brain? Research support from NIH NS1288.

Poster Session 1 | Poster Wall 78 | Label: PS1.078

Category: Evolution and development

Diversity and Evolution of Neuronal Cell Types in Cephalopods

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Coleoid cephalopods are soft-bodied, marine invertebrates that evolved complex brains and sophisticated behaviors, such as dynamic camouflage, independently of vertebrates. Thus, analyzing the cell types and circuits in cephalopod brains provides a fascinating opportunity to study convergent evolution and identify common (or divergent) principles of sensory and motor computations. To elucidate the molecular basis of cephalopod brain evolution, we are generating a whole-brain atlas of two species of cuttlefish, Sepia officinalis and Sepia bandensis, by profiling the cell type diversity of their brain using single-nucleus RNA sequencing and spatial transcriptomics methods.

By leveraging published transcriptomic datasets from other cephalopod species (e.g., Octopus vulgaris, Octopus bimaculoides), we are conducting detailed cross-species comparisons to understand the conservation of cell types and the emergence of novel cell types that might underlie species-specific adaptations and behaviors. Moving beyond comparisons between cephalopods, we plan to include more species in the future to elucidate the emergence of the complex cephalopod nervous systems from simpler neural structures found in molluscan or protostome ancestors. Ultimately, by exploring the evolutionary trajectory from simpler neural structures to the sophisticated cephalopod brain, we hope to shed light on fundamental principles and evolutionary constraints driving the evolution of neural systems.

Poster Session 1 | Poster Wall 79 | Label: PS1.079

Category: Evolution and development

Evolution of olfactory cell types and receptors across mosquito antennae

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The sense of smell is a critical interface between organisms and their environment, allowing animals to detect and respond to the odor of food, mates, predators and other resources. We might therefore expect the genes and cells that underlie odor detection to evolve rapidly. Indeed, olfactory receptors are often the most rapidly evolving genes in animal genomes, blinking on and off across species as genes are duplicated, deleted, and evolve novel tuning. Much less is known about the evolution of the sensory neurons in which these receptors are expressed. For example, mosquitoes are an ancient family of flies that date back 180 million years. Their odorant receptors evolve rapidly, with fewer than 10% of genes having 1-to-1 orthologs across species, yet all species appear to have the same number of sensory neuron cell types. Are these cell types conserved or do they undergo a rapid birth-death process similar to receptors? If they are conserved, how do spatial patterns of receptor expression and coexpression evolve on top of this conserved cellular substrate? I will address these questions with a cross-species analysis of snRNAseq data from antennal neurons in three species of disease-vector mosquitoes: the yellow fever mosquito (Aedes aegypti), the Asian tiger mosquito (Aedes albopictus), and the West Nile Virus mosquito (Culex quinquefasciatus). Each of these species has 120-180 odorant receptors but only ~60 types of olfactory sensory neurons. I will attempt to resolve patterns of cell type homology and receptor expression across species and uncover the tempo and character of peripheral olfactory evolution in a cellular context.

Poster Session 1 | Poster Wall 80 | Label: PS1.080

Category: Evolution and development

Visual ecology and feeding behavior drive brain size expansion across hawk and silk moths

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When it comes to the processing power of the nervous system, bigger is generally better. However, brain size evolves under a trade-off between the advantages of increased size and the metabolic costs of more neural tissue, all within the constraints of developmental patterning. Under general size constraints functional regions of the brain may also face size tradeoffs with each other. To date our knowledge of the patterns and selective forces of brain size evolution is derived from data on a small number of clades, largely vertebrates. Thus, our general understanding of the forces determining brain size and architecture can be enriched with data from additional clades. While butterflies and moths (Lepidoptera) have received much attention in neuroethology, phylogenetics, and even trait evolution, little is known about brain evolution in this group. Here we use volumetric data gathered from micro-CT images of whole heads to assess brain size scaling in moths of the superfamily Bombycoidea, a group with a well-resolved phylogeny, and a large range of body sizes and ecological niches. The diversity in size and behavior of these moths allows us to compare brain size and architecture across diel activity patterns, flight and visual capabilities, and adult feeding capacity. Preliminary analyses suggest that selection on visual ability required for navigation and adult feeding behavior determine total brain size in this group, but that investment in vision may occur as a trade-off with olfactory regions.

Poster Session 1 | Poster Wall 81 | Label: PS1.081

Category: Evolution and development

Towards the anatomical blueprint of the insect brain – brain morphology across the insect phylogenetic tree

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Insect brains show a shared anatomical ground pattern with clearly homologous brain regions across all insect groups investigated to date. This is remarkable, given the enormous diversity of insects, which have evolved from a common ancestor more than 450 million years ago. The insect brain consists of circa 40 distinct neuropils. Highly structured interconnections between brain regions couple neural circuits across neuropil boundaries. We hypothesize that neuropils that are strongly functionally linked to one another should remain linked across evolutionary times, i.e. evolve in concert. In contrast, regions that operate more independently should evolve independently (mosaic brain evolution) – effects that should be visible at the level of brain region size. We have thus started to obtain whole-brain anatomical data from species across the insect phylogeny, spanning more than 13 orders and 30 species. We have used both immunohistochemistry combined with confocal microscopy as well as X-ray based imaging techniques as the basis for reconstructing 3D neuropil models. Quantitative analysis of the resulting neuropil data has revealed that within lepidoptera, the regions of the central complex (CX), the lateral complex (LX) and the small subunits of the anterior optic tubercles (AOTU) show remarkable little interspecies variation, while the mushroom bodies, optic lobes and antennal lobes change much more strongly between species. This indicates that the regions referred to as compass neuropils (CX, LX, AOTU) form a tightly linked group of neuropils, with little room, or need, for substantial modifications during evolution. In contrast, all other brain regions appear to have the capacity to respond to ecological demands by readily adapting their size, and thus their information processing capacity. We are currently expanding these analyses to include up to 100 species to test whether this pattern provides a ground plan found throughout insects.

Poster Session 1 | Poster Wall 82 | Label: PS1.082

Category: Evolution and development

Rapid evolution of sociality is paralleled by changes in the activation pattern of the brain social decision-making network in zebrafish

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The social brain hypothesis (SBH) is a theory in evolutionary biology that posits that the social environment is a major driver of brain and cognitive evolution. This hypothesis has focused on brain size as a proxy of brain computational power (i.e. cognitive potential), and has mainly used a comparative approach across species varying in their sociality. Therefore, experimental methods, which allow to establish causality between social and brain evolution, and more detailed measures of brain function beyond brain size are still missing in the field. Here, we experimentally tested the effects of the evolution of sociality on brain function. An artificial selection experiment for high and low sociality lines was performed in zebrafish using a two-choice paradigm test (i.e., videoplaybacks of a shoal vs moving dots). After only three generations (F3), the high-sociality line started to diverge significantly from the control lines, exhibiting a higher social preference. In the fifth generation (F5), we investigated whether the divergente phenotypes had different neuronal activation patterns in response to a social stimulus. We used pS6 as a molecular marker of neuronal activation and analysed a set of nuclei of the social decision-making network (SDMN). Neural activation patterns reveal differences between the lines in distinct brain nuclei. Our results, indicate a significant decrease in cell activation in the Vv (putative homologue of the lateral septum) in the high sociality line.This nucleus has a large population of inhibitory cells, whose reduced activity is linked to social fear contagion in zebrafish, suggesting that heightened sociality is accompanied by a decrease of activity of these inhibitory cells. Overall, we demonstrate that sociability is a trait than can be selected and the divergent social phenotypes are implemented by different neuronal network activation patterns in the SDMN.

Poster Session 1 | Poster Wall 83 | Label: PS1.083

Category: Evolution and development

Development and structural dependence on binocularity of "innate" escaping responses in a diurnal rodents, the Octodon degus

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The way an animal orient and conjugate its eyes plays a crucial role in shaping how it will perceive and attend to the surrounding world. Comparative studies have revealed a strong correlation between the configuration of an animal's visual field and its visual ecology and behavior. For instance, highly convergent eyes and orbits provide wide binocularity to hunting as well as to dexterous nocturnal species. Conversely, species with lateralized eyes and orbits enjoy a panoramic view, useful for detecting predators. Eye position also influences the development of retinal specializations, the extent of ipsilateral retinal projections, and the development of cortical areas. However, how proper eye orientation is coordinated with the maturation of the skull, the retina and behavior remain largely unexplored. Here, we show that the eyes and orbits of the rodent species Octodon degus converge during postnatal development, to expand the binocular field from approximately 20° at P5 to around 60° in adults. In parallel, regions of higher-acuity in the retina such as the area centralis and visual streak develop progressively from an initial, relatively uniform retinal ganglion cell distribution. Additionally, we found that escaping from aversive stimuli in the upper binocular field aligns with the binocular enlargement and development of the retinal specializations. These findings illustrate how postnatal changes in eye and orbit orientation collectively contribute to the configuration of the binocular visual field and correlate with changes in retinal topography and behavior. Moreover, they provide valuable insights to understand the mechanisms driving the diversity of form and function of binocular vision during mammalian evolution.

Poster Session 1 | Poster Wall 84 | Label: PS1.084

Category: Metabolism, biological rhythms and homeostasis

RNA expression analysis of individual accessory medulla neurons of Rhyparobia maderae using single nucleus RNA sequencing

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Organisms evolved endogenous circadian clocks to anticipate dusk and dawn during the daily 24h light-dark cycle. Transcriptional-translational feedback loops (TTFLs) in clock gene expression were described as clockwork of single circadian clock cells. While the fundamental principle of a TTFL-based molecular clockwork is well-established in mammals and insects, it is still not understood how it governs rhythms in physiology and behavior.

We focus our research on the large, long-lived Madeira cockroach Rhyparobia maderae, a circadian model species, well suited to electrophysiological and behavioral analysis. Transplantation experiments identified the accessory medulla (AME) with pigment-dispersing factor (PDF) containing neuropeptidergic clock neurons as circadian pacemaker governing rest-activity cycles. Previous immunocytochemical and electrophysiological experiments revealed that AME clock neurons express great heterogeneity in colocalized neuropeptide and neuropeptide/neurotransmitter receptors expressions, and, thus in functional connectivity. However, mass-stainings make it difficult to reveal individual neuronal branching patterns and to predict respective functions in light entrainment, local, or output pathways of the circadian clock network.

To decipher functional connectivity of PDF clock neurons, we started an extensive RNA expression analysis of AME clock neurons. We opted to sequence roughly 1000 nuclei with high read-depth, from frozen tissue samples, that were pooled across different time points, with droplet-based single nucleus RNA sequencing. We focus on searches for clock genes, neurotransmitter pathways, neuropeptide precursors, and neuropeptide receptors in single AME clock cells. We expect to find different clusters among the AME/PDF cells, with characteristic neurotransmitters/neuropeptide-combinations that allow us to distinguish light entrainment from local, and output clock pathways predicted by immunocytochemical studies.

Poster Session 1 | Poster Wall 85 | Label: PS1.085

Category: Metabolism, biological rhythms and homeostasis

Circadian and seasonal physiology of glassfrogs in the light of climate change

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Biological rhythms are a core component of organisms' physiology and behavior. Circadian rhythms play an important role in organisms' fitness, as they confer the ability to anticipate and prepare for cyclic environmental changes. However, climate change has disturbed the onset of seasons, as photoperiod information is no longer an accurate cue to predict temperature, rainfall, humidity, and other resources. Amphibians are particularly affected by climate change, as their life cycle depends on water and land conditions, thus understanding the effects of the circadian misalignment created by climate change can be crucial to this group. However, little is known about the organization of the circadian system in amphibians. Glassfrogs have a transparent ventrum and while sleeping, they conceal their blood in the liver, becoming even more transparent. Glassfrogs are emerging as a possible new organism model for physiology research in amphibians due to recent genomic tools and characteristic transparency. We characterize daily hormonal rhythms using water-born samples and recorded activity-rest rhythms in glassfrogs. To understand the effects of circadian misalignment and climate change in amphibians, we will identify circadian oscillators in glassfrogs, characterize seasonality in the field, and test for potential desynchrony with future climate conditions. Understanding the daily and seasonal aspects of the physiology and behavior of glassfrogs might contribute to understanding how climate change will impact amphibians' survival and evolution.

Poster Session 1 | Poster Wall 86 | Label: PS1.086

Category: Metabolism, biological rhythms and homeostasis

Peptidergic and aminergic modulation of Insulin-Producing Cells in Drosophila

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Insulin plays a crucial role in regulating metabolic homeostasis and other important bodily functions in vertebrates and invertebrates. In Drosophila melanogaster, insulin is predominantly released by Insulin-Producing Cells (IPCs) in the central brain and acts as a neuromodulator within the nervous system and on tissues throughout the body. Hence, IPC activity and therefore insulin release must be dynamically adjusted to internal demands. Here, we characterized the functional inputs to IPCs using single-nucleus RNA sequencing analysis, anatomical receptor expression mapping, and in vivo electrophysiology and calcium imaging combined with optogenetics. Our comprehensive receptor profile revealed that the IPCs express a broad variety of receptors for neuromodulators and classical neurotransmitters. Interestingly, the IPC population exhibited a heterogeneous receptor profile, suggesting that individual IPCs could be modulated differentially. This was validated by electrophysiological recordings we performed in IPCs while activating key modulatory neuron populations. Some modulatory inputs had heterogeneous effects on individual IPCs, such that they excited one subset of IPCs, while inhibiting another. Calcium imaging experiments across the IPC population uncovered that these heterogeneous responses occurred simultaneously to the same input. In addition, certain modulatory inputs shifted the overall IPC population activity towards an excited state, while others shifted the IPC activity towards inhibition. Taken together, we compiled an extensive, multi-level framework for neuromodulation of the insulinergic system in Drosophila and revealed a high level of regulatory flexibility.

Poster Session 1 | Poster Wall 87 | Label: PS1.087

Category: Metabolism, biological rhythms and homeostasis

Nutritional state-dependent modulation of Insulin-Producing Cells in Drosophila

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Insulin plays a key role in regulating metabolic homeostasis across vertebrate and invertebrate species. Drosophila Insulin-Producing Cells (IPCs) are functional analogues to mammalian pancreatic beta cells and release insulin directly into circulation. IPC activity is modulated by nutrient availability, circadian time, and the behavioral state of animals. To investigate the in vivo dynamics of IPC activity in the context of metabolic homeostasis, we quantified effects of nutritional and internal state changes on IPCs using electrophysiological recordings. We show that the nutritional state strongly modulates IPC activity. IPCs were less active in starved flies than in fed flies. Refeeding starved flies with glucose significantly increased IPC activity, underscoring that IPCs are regulated by hemolymph sugar levels. In contrast to glucose feeding, glucose perfusion had no effect on IPC activity. This was reminiscent of the mammalian incretin effect, in which ingestion of glucose drives higher insulin release than intravenous glucose application. Contrary to IPCs, Diuretic hormone 44-expressing neurons in the pars intercerebralis (DH44PINs) significantly increased their activity during glucose perfusion. Functional connectivity experiments demonstrated that glucose-sensing DH44PINs do not affect IPC activity, while other DH44Ns inhibit IPCs. This suggests that populations of autonomously and systemically glucose-sensing neurons are working in parallel to maintain metabolic homeostasis. Ultimately, metabolic state changes affect animal behavior. In support of this idea, activating IPCs had a small, satiety-like effect in starved flies, resulting in reduced walking activity, whereas activating DH44Ns strongly increased walking activity. Taken together, we show that IPCs and DH44Ns are an integral part of a sophisticated modulatory network that orchestrates glucose homeostasis and adaptive behavior in response to shifts in the metabolic state.

Poster Session 1 | Poster Wall 89 | Label: PS1.089

Category: Metabolism, biological rhythms and homeostasis

Remote chemical command from the female to male clock induces and synchronises insect circadian rhythms

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To extract any adaptive benefit from the circadian clock that dictates the daily time of behavioural activity of every animal, the clock needs to be synchronised to the 24-hour day-night cycles. Clock-generated internal oscillations are matched with the external time by light and temperature cycles. We examined if it is a general property of the brain's circadian clock to recognise social interactions as an external time-giver. Sociosexual interactions with the opposite sex are universal, prevalent even in the lives of solitary animals – presenting a prototype to assess the impact of elemental social interactions on timekeeping. We employed male Spodoptera littoralis moth as our model, leveraging its adult life singularly dedicated to sex, and hence providing an ideal context to explore the impact of sociosexual cues on the circadian clock. Here, for the first time, we identify specific olfactory cues responsible for social entrainment, and reveal surprisingly strong influence of remotely occurring, pheromone-mediated sociosexual interactions on the circadian rhythms of a solitary insect. Males' free-running rhythms are induced and synchronised by the sex-pheromone that the female releases in a rhythmic fashion, highlighting a hierarchical relation between the female and male circadian oscillators. Our finding of daytime-dependent lasting impact of pheromone on male's courtship efficacy implies that circadian timing in moths is a trait under the distinct evolutionary force of sexual selection. Within the sex-pheromone blend, we unearth specific components that lack mate-attractive property but exert powerful circadian effects, providing rationale for their continued retention by the female. We show that such volatiles can be shared across sympatric moth species and trigger communal synchronisation. Thus, our results suggest that sex-pheromone released by female moths modify males' behavioural activity rhythm to ensure synchronised timing of mating.

Poster Session 1 | Poster Wall 90 | Label: PS1.090

Category: Metabolism, biological rhythms and homeostasis

Stress Responsiveness in laboratory-bred and wild (urban and rural trapped) Rattus norvegicus: Consideration of adaptation and habitat influences

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Although an animal's species-specific umwelt is important to consider in neurobiological research, featureless environments are often used in laboratory investigations. Previous research in our lab indicates that wild rats exhibited traditional markers of stress including heavier adrenal glands and higher corticosterone fecal metabolite levels than lab rats (Jacob et al., 2022). Considering that emotional stress is generally associated with negative health outcomes, we reported that wild rats had heavier brains and higher neuronal density in the cerebellum in comparison to the lab rats (suggestive of a healthier neural profile in wild rats). In the current study, habituation rates of corticosterone fecal metabolites in wild rats trapped in the city of Richmond VA USA were compared to lab-bred male and female rats (n=3 per group; N=12). Initial results indicate that the wild rat corticosterone metabolite levels decreased across the five days in captivity; however, the lab rat counterpart data are still being analyzed. In an additional phase of the investigation, wild rats were trapped in a rural setting in Rockbridge County, VA USA to compare to urban-trapped Richmond, VA wild rats, as well as and laboratory-bred rats. Results indicate that the urban-wild rats' fecal corticosterone metabolites were significantly higher than the rural and laboratory R. norvegicus cohorts.

Poster Session 1 | Poster Wall 91 | Label: PS1.091

Category: Metabolism, biological rhythms and homeostasis

The neuronal membrane as circadian posttranslational feedback loop oscillator

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Behavior of organisms is organized in circadian (~ 24 h) patterns: sleeping, feeding, or mating occur at specific zeitgeber times (ZTs) per day. Such regular, predictive rhythms enable anticipation and synchronization of behavior within and between species. They are driven by endogenous clocks that are entrained to daily zeitgebers like the light-dark cycle.

In insects, the central "master clock" is a neural network in the brain, while peripheral clocks exist in sensory neurons and other tissues. The current model in chronobiology frames the clock as hierarchical, autonomous transcription/translation feedback loop (TTFL) that drives the circadian expression of clock proteins, controlling all circadian oscillations in an organism. Instead, we propose that circadian clocks are based on a coupled system of TTFL and posttranslational feedback loop (PTFL) oscillators.

We use electrophysiological analysis of olfactory receptor neurons (ORN) in the antennae of male hawkmoth ([i]Manduca sexta[/i]) in combination with pharmacology and molecular genetics. The ORNs are endogenous circadian clock neurons. Their spontaneous ultradian spiking shows circadian modulation with maximal spiking during the nocturnal hawkmoth´s activity phase. With application of various antagonists and agonists, in combination with molecular genetics, we search for ion channels involved in ZT-dependent rhythm generation. Preliminary work suggests that, rather than TTFL-dependent control, PTFL-dependent oscillators associated with plasma membrane-dependent signaling cascades generate the observed multiscale oscillations. Apparently, membrane potential oscillations are based on cyclic nucleotide- and calcium-dependent ion channels, which controls sensitivity and kinetics of ORNs. Our current experiments search for links between these rhythms at timescales spanning several log units.

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Poster Session 1 | Poster Wall 92 | Label: PS1.092

Category: Metabolism, biological rhythms and homeostasis

The Potential Impact of Environmental Complexity on Cerebrovasculature in Wild and Laboratory R. norvegicus

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Vascularization is crucial for meeting the brain's metabolic demands. Previous investigations of rats in enriched environments indicate a positive correlation between environmental complexity and cortical microvascular density (MVD; Sirevaag et al., 1988). Recently, our lab observed increased neural cell counts and elevated stress hormones in wild-caught rats from Richmond, VA compared to weight-matched Long-Evans laboratory rats (Jacob et al., 2022). Building on these initial results, we compared MVD in wild female rats (R. norvegicus) with laboratory-bred R. norvegicus (n=5 each group) in the current study. Focusing on brain regions implicated in emotional and cognitive functions, namely the hippocampus and anterior cingulate cortex (ACC), we hypothesized that wild rats would have higher MVD scores due to the complexity of their natural habitat compared to their laboratory counterparts. Fresh brains were post-fixed upon removal and sectioned at 40 μm for immunocytochemistry to quantify mineralocorticoid receptors, an antibody known to mark blood vessels. Results indicated that the wild rats had approximately 60% higher visible immunoreactive vasculature scores than the lab rats in the dentate gyrus (DG) of the hippocampus ($p = .016$) and approximately 260% higher scores than the lab rats in the ACC ($p = .004$). Because natural habitats are complex with both engaging and threatening stimuli, the second phase of this study examined the impact of laboratory-induced environmental enrichment on cerebrovasculature via immunofluorescence procedures targeting Collagen IV, a marker for the basal lamina of blood vessels. Analyses of Collagen IV immunoreactive tissue in the DG are ongoing. Together, these results will help elucidate the influence of complex, dynamic environments on the brain's MVD, with translational potential for cerebrovascular disease outcomes.

Poster Session 1 | Poster Wall 93 | Label: PS1.093

Category: Metabolism, biological rhythms and homeostasis

Swamp Minds: weakly electric fish Petrocephalus degeni balance brain cell proliferation and apoptosis in hypoxia

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Brain dysfunction during warming is triggered by a cascade of pathological changes stemming from oxygen limitations in aquatic habitats, significantly impacting the ecology, behavior, and physiology of fishes. We investigated apoptosis and compared it to brain cell proliferation in the brain of the weakly electric fish Petrocephalus degeni, shedding light on their adaptation mechanisms to the extreme hypoxia they experience in their natural habitat in swamps in Uganda. These fish inhabit an environment with minimal oxygen availability yet exhibit typical mormyrid brain characteristics, i.e. a very large brain with a gigantocerebellum. Thirty-three individuals were collected, with 11 sacrificed in the field and 22 exposed to artificial hypoxia or normoxia in a laboratory setting. PCNA analysis revealed that swamp-dwelling field fish displayed the highest rates of brain cell proliferation, with normoxic fish significantly outperforming hypoxic lab counterparts. Additionally, a TUNEL analysis staining apoptotic cells showed a higher apoptotic rate in fish in the hypoxic lab conditions than in fish from the swamp or kept under normoxic conditions in the lab. Our results suggest strong effects of both, oxygen regime and captivity, on the balance between brain cell proliferation and apoptosis, and remarkable resilience of the brains of these fish under naturally hypoxic conditions.

Poster Session 1 | Poster Wall 94 | Label: PS1.094

Category: Metabolism, biological rhythms and homeostasis

Molecular Mechanisms Mediating the Effects of Social Cues on Biological Clocks

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While the effect of photoperiod on biological rhythms is widely studied, how social cues influence circadian rhythms in vertebrates is poorly understood. We investigated whether light or social cues are a stronger influence on reproductive calling behavior and mRNA expression of core clock genes in the brain of green treefrogs (Hyla cinerea). We collected male frogs from a wild population and housed them individually in soundproof acoustic chambers. Frogs were exposed to either a normal (LD) or reverse (rLD) 12:12 h light: dark cycle and an acoustic treatment of either chorus (a recording of a naturally breeding population), tones (positive sound control), or silence from 0100-0500 h on each of 7 consecutive nights. The timing of the acoustic stimulus corresponded to the middle 4 hours of the dark phase for the LD photoperiod group and the middle 4 hours of the light phase for the rLD group. Frogs were euthanized and brains collected every 4 h for 24 hours for use in quantitative PCR (qPCR). As expected, the total number of reproductive calls produced by frogs hearing the chorus acoustic stimulus was significantly higher than that of frogs hearing tones or silence.

Poster Session 1 | Poster Wall 95 | Label: PS1.095

Category: Metabolism, biological rhythms and homeostasis

Regulation of Appetite by Dietary Macronutrients: Unraveling the Host-Microbiome Connection

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All metazoan guts harbor complex commensal communities, from around a dozen bacterial species in Drosophila to hundreds in humans. Gut microbiota, malleable by an animal's dietary history, can conversely influence the host's feeding behavior. Here, we condition flies with diets containing varying levels of protein and sugar to investigate the impact of dietary history on the interaction between commensal gut bacteria and feeding adaptation in Drosophila. We find that fly appetite increases with dietary protein, dependent on total gut bacteria content, and enhanced by metformin, a drug known to promote the growth of short chain fatty acid (SCFA)-producing gut bacteria. Further qPCR analysis of the two main commensal bacteria in Drosophila shows that Lactobacillus, a potential source of SCFA butyrate, emerges as dominant at a high protein level, while Acetobacter, known for producing SCFA acetate, dominates at a low protein level. Mono-association of germ-free flies with Lactobacillus or Acetobacter increases their food intake, with highest intake observed in those associated with Lactobacillus and conditioned by a high-protein diet. Additionally, mutant Acetobacter and Lactobacillus strains unable to produce acetate and butyrate, respectively, have lesser effects. Finally, we find that adding either acetate or butyrate to the conditioning diets increases appetite of germ-free flies, recapitulating the appetitive effect of Acetobacter and Lactobacillus, respectively. Our findings suggest that protein-enriched diets enhance host appetite by promoting the interaction between commensal bacteria and the host, with bacterial SCFAs as a conduit for the interaction.

Poster Session 1 | Poster Wall 96 | Label: PS1.096

Category: Metabolism, biological rhythms and homeostasis

Local sleep in songbirds: Different simultaneous sleep stages in zebra finches

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Traditionally, wakefulness and sleep have been viewed as distinct, all-encompassing brain states. However, recent research in rodents and humans challenges this notion by shedding light on the local regulation of sleep stages. Our study examines the local regulation of sleep stages in zebra finches, focusing on the avian pallium.

Using electroencephalogram (EEG) and local field potential (LFP) recordings across multiple sites within the avian pallium, we uncovered new insights. Contrary to the conventional assumptions, we found that sleep stages are not uniform across the entire pallium. Instead, we observed significant variations in slow wave sleep (SWS) percentages and durations, particularly in deeper mesopallium sites compared to surface sites.

We scored sleep into stages independently in each electrode site and quantified the concurrent mismatches between the sleep scores across the electrodes. Our analysis revealed that sleep stages do not occur simultaneously across all electrode sites for a major portion of time bins.

These findings challenge the established notion of global sleep regulation and underscore the importance of local mechanisms in governing sleep dynamics in the avian brain.

Poster Session 1 | Poster Wall 97 | Label: PS1.097

Category: Methods and education

Real-time analysis of large-scale neuronal imaging enables closed-loop investigation of neural dynamics

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Large-scale imaging of neuronal activities is crucial for understanding brain functions and behavior. However, it is challenging to analyze large-scale imaging data in real time, preventing closed-loop investigation of neural circuitry. Here we develop a real-time analysis system with a field programmable gate array–graphics processing unit design for an up to 500-megabyte-per-second image stream. Adapted to whole-brain imaging of awake larval zebrafish, the system timely extracts activity from up to 100,000 neurons and enables closed-loop perturbations of neural dynamics.

Poster Session 1 | Poster Wall 98 | Label: PS1.098

Category: Methods and education

RoboFish: A Novel Robotic System for Understanding Weakly Electric Fish Dynamics

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Mormyrid weakly electric fish use electric organ discharges (EODs) for orientation, species recognition, and group coherence. However, decoding how specific interactions among shoal members influence overall group behavior remains a challenge. We introduce a novel biomimetic robotic system designed for interaction and communication with weakly electric fish, featuring a robotic fish that emits natural, temporal EOD patterns in response to the electric signals from living fish. This allows us to observe the reactions of real fish when confronted with predetermined, interactive behaviors exhibited by the robot.

Experiments are performed with one robot and multiple fish in a 120 cm x 100 cm tank equipped with four pairs of differential electrodes used to record the electric behavior of the group. The electrodes are connected to a custom interface board, the ElectroFish Interface (EFI), which continuously records all EODs. The EFI runs a neural network on specialized hardware to localize EODs in real-time with very low latency (less than 10 ms).

The robotic fish carries an electrode pair that allows it to generate electric pulses, for instance, as a response to EODs emitted by specific individuals in the group.

A video camera monitors the tank under blue moonlight LEDs. Using computer vision, the ElectroFish system continuously tracks fish positions, enabling the robot to execute various locomotive behaviors, such as approaching, leading, passing, and following, which can be combined with electric behavioral modes as needed. This allows for dynamic, real-time interactions between the robot and a target fish.

Our system has the ability to accurately track and electrically interact with live fish, allowing us to test a range of interactive behaviors in a standardized manner. This advances our understanding of weakly electric fish and lays the groundwork for future aquatic social system studies.

Poster Session 1 | Poster Wall 99 | Label: PS1.099

Category: Methods and education

A practical course, designed by students, using Backyard Brains equipment

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During a 4-week project period, seven students from a science-focused liberal arts college designed a practical course in neurobiology. Several of these students took neuroscience courses in the past. They had access to Backyard Brains equipment that enabled them to make electrophysiology recordings. A course booklet with background information, instructions and questions was developed, in which the students also had to give rational for their choices. They picked five topics; (1) Introduction to neurobiology and electrophysiology, (2) conduction velocity, (3) EEG and data analysis, (4) neuropharmacology and (5) application in neuroscience.

The proposed lab sessions were tested. Most instructions came directly from the Backyard Brains website, but were occasionally altered for clarity or because students obtained better results with slight changes in the procedures. For the neuropharmacology experiment, the students choose a grasshopper vision experiment. This experiment was not designed as neuropharmacology experiment by the Backyard Brains company and without instructions, the students struggled to figure out dosages of alcohol and nicotine. In general, the students liked the Backyard Brains equipment but had some issues getting software to run on their computers and thought the Spikerboxes were fragile. They also indicated that the experiments were perhaps a bit simple for a college level course.

In a subsequent project period the practical course was tested by seven other students. They revised some of the protocols for clarity. Like the previous group, they struggled with dosages in the neuropharmacology experiment. In addition, they indicated that experiments 3 and 5 were too short to fill a full day in the lab. From their report it also became apparent that the instructions for the conduction velocity experiment need to be clearer, as they misinterpreted the setup and were unable to get recordings.

Poster Session 1 | Poster Wall 100 | Label: PS1.100

Category: Methods and education

CRISPR/Cas9 mediated targeted genome editing in the parthenogenetic stick insect Medauroidea extradentata

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Stick insects have long been used as experimental animals to investigate the neural basis of locomotion and belong to the most thoroughly studied creatures in that respect. Experimental work would markedly profit from an in-vivo visualization or identification of neuron classes within the central nervous system, for which molecular genetic interventions are needed. However, genetic tools for this order of insects have not been implemented, limiting further approaches towards unravelling neural mechanisms underlying motor control, even though their parthenogenetic life cycle offers unique advantages. Here, we optimize and apply Clustered Regularly Interspaced Short Palindromic Repeats/Cas associated protein 9 (CRISPR/Cas9; Jinek at al. 2012) as a technique to modify the genome of the stick insect Medauroidea extradentata.

For proof of principle, we targeted genes involved in the ommochrome pathway of eye pigment transport and synthesis (cinnabar, white, cardinal; Summers et al. 1982), performing microinjection of eggs within 24h after oviposition to generate knockout (KO) mutants. Cinnabar and white KOs resulted in distinct eye and cuticle colour phenotypes. Homozygous cinnabar KOs showed a phenotype with white eyes and cuticle, but died during the process of hatching. However, one mosaic animal successfully reached adulthood and gave rise to viable homozygous KO hatchlings, therefore showing that induced CRISPR/Cas9 events are stable and can be transmitted to the next generation by parthenogenetic mechanisms. In contrast, white KO was lethal to developing embryos, which displayed a completely unpigmented phenotype.

In conclusion, we showed that CRISPR/Cas9 can be successfully applied on the genome of M. extradentata by creating mutants with different phenotypes. This genetic toolbox can now be implemented to create genetically modified lines to enable further unravelling of motor control using state-of-the-art methods in an upcoming parthenogenetic model organism.

Poster Session 1 | Poster Wall 101 | Label: PS1.101

Category: Methods and education

Novel methods for the study of insect social behavior

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Paper wasps (genus Polistes) form complex social dominance hierarchies, show diverse and rich social behaviors, and impressive cognitive feats. Within this group, multiple species have evolved facial color patterns that signal individual quality (e.g., P. dominula) and, in at least one species, individual identity (P. fuscatus). P. fuscatus can infer the winner or loser of a third-party fight simply through observation and keep long term memory of individual social interactions. These behaviors have been studied mainly through observations in dyadic behavioral trials, as experimentally manipulating the behavior of a social partner is a challenging task.

Here, we adapted a detailed insect 3D scanning procedure and combined it with motion capturing of freely behaving animals to virtualize natural behaviors like fights very accurately. By interchanging movement patterns, textures, and even the insect species itself, we can present P. fuscatus with artificial social scenes, for example, showing rank reversals or fights with different levels of aggressive intensity. This method will allow us to study the wasps' internal model of the whole nest hierarchy in future experiments. In addition to showing videos only, we can combine the virtually behaving wasps with a trackball VR setup and allow animals to interact with virtual social partners in a tightly controlled way. Even though it is only applied to paper wasps here, the presented pipeline opens many novel possibilities for the study of social behaviors and even their neural representations in diverse insect species.

Poster Session 1 | Poster Wall 102 | Label: PS1.102

Category: Methods and education

Clearing the path for molecular imaging in the cichlid brain

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Neurobiological investigations in non-model organisms benefit from innovative techniques to explore the complex architecture and functions of the brain. RNA in situ hybridizations are providing access to neuronal subpopulations (via genetic markers), are helping to demarcate neuroanatomical boundaries, and are also being used to label functionally active neurons by virtue of their immediate-early gene (IEG) expression. Here we adapted the highly sensitive Hybridization Chain Reaction (HCR) method to the adult cichlid brain. HCR is a powerful method for molecular imaging due to its multiplexability (up to 9 probes per specimen), specificity, and signal amplification. Its effectiveness, however, largely depends on the optical accessibility of the tissue. In this study, we developed a workflow to visualize IEGs and other molecular targets in whole-tissue cleared brains of two different cichlid species, Lamprologus ocellatus and Astatotilapia burtoni, by means of confocal and light-sheet microscopy. This robust and versatile approach is contributing to our understanding of the functional neuroanatomy of the cichlid brain and can be easily extended to other species.

Poster Session 1 | Poster Wall 103 | Label: PS1.103

Category: Methods and education

Do it yourself: Creating custom-made 3D brain surface models and brain matrices for sectioning using photogrammetry and three-dimensional printing technology

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Neuroethologists study many non-model organisms, encompassing a diverse range of species. Key to advancing our understanding of neural mechanisms across these species is the development of techniques for precise and standardized histological brain analysis. Here, we present a novel cost-effective approach combining photogrammetry and three-dimensional printing technology to generate 3D brain surface models and produce species-specific brain matrices for precise and reproducible trimming, blocking and sectioning. Using this strategy, we successfully generated a 3D brain surface model and a brain matrix for Seba's short-tailed fruit bat, Carollia perspicillata. The 3D brain surface model was generated using the open-source 3D graphics suite Blender and its commercially available, but inexpensive photogrammetry plug-in Snapmesh. As basis for the 3D-reconstruction a set of high-quality photos of brains of two different male individuals was used, resulting in an "average" surface model of C. perspicllata's brain. Here, photogrammetry offers a viable cheap alternative to techniques requiring access to costly 3D- or CT-scanners. This holds promise, since these models could potentially aid the alignment of brain images in a common reference space for comparative studies. The brain matrix was modeled in Blender, and printed using a 3D printer. Our brain matrix facilitates and standardizes trimming or blocking of brains, and free-hand slicing of discrete regions of the brain of C. perspicillata in both, the sagittal and coronal plane. Finally, our workflow is tissue-loss-free, meaning no animal is sacrificed for the generation of a brain surface model or brain matrix alone. In sum, our workflow could benefit researchers globally, especially those with limited funding or working with particularly valuable specimen, by providing a viable, affordable, and tissue-loss-free alternative for the generation of 3D brain surface models and brain matrices.

Poster Session 1 | Poster Wall 104 | Label: PS1.104

Category: Methods and education

Classroom Assessment of Crescent Loom, A Simulation for Teaching Neural Circuits and Behavior

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Digital simulations are regularly used to teach concepts related to neurophysiology, but relatively few simulations successfully convey how a neural circuit contributes to embodied behavior. Crescent Loom's Connectome Explorer is an online simulation that prompts students to use electrophysiology and optogenetics tools to deduce the connectivity of a neural circuit and relate features of its structure to function. Here I present some assessment data on the implementation of Crescent Loom in undergraduate Animal Behavior and Introductory Neuroscience courses at two small liberal arts colleges in the United States. Students took a short quiz before and after completing a Crescent Loom activity in class; the post-quiz also included Likert Scale items that assessed student perception and usability of Crescent Loom. Preliminary analyses reveal that Crescent Loom had mixed effectiveness in teaching concepts related to synaptic connectivity and features of pacemaker neurons, but student learning gains did not correlate with how they rated the usability of Crescent Loom. Likert Scale survey questions indicated that students enjoyed using Crescent Loom. Student self-report of enjoying playing video games in their spare time did not significantly affect their perceptions of Crescent Loom's usability. Overall, Crescent Loom appears to be a promising and low-cost way to teach students about how neural circuits generate behaviors.

Poster Session 1 | Poster Wall 105 | Label: PS1.105

Category: Methods and education

Light microscopy-based dense connectomics in invertebrates

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Animal behaviors are mediated by expansive networks of cellular and molecular components. Mapping the structure of these networks is essential to a complete understanding of the neural basis of behavior. The synaptic structure of densely labeled cellular networks can be reconstructed from nanoscale resolution image volumes acquired using electron microscopy (EM). However, EM does not capture molecular labels and requires specialized equipment not available in many research settings. Light microscopy enables molecular labels to be identified in large image volumes using commonly available instruments, but resolution and tissue contrast limitations have prohibited cell-type-specific molecular localization and synaptic connectivity mapping in densely labeled cellular networks. Recent work in mammals has demonstrated that new techniques in expansion microscopy can be utilized to reconstruct densely labeled circuits with synaptic resolution and molecular contrast by acquiring nanoscale brain image volumes with EM-like ultrastructural contrast using conventional light microscopes. Here, we adapt and optimize this approach for invertebrates, with an initial focus on Drosophila. We utilized this approach to rapidly acquire whole brain ultrastructure image volumes with effective lateral resolutions of 10 nm. We demonstrate our ability to simultaneously label brain ultrastructure and molecules of interest, segment individual neurons, and to identify cellular organelles and synaptic connections. This work enables dense circuit connectomics with molecular contrast to be utilized to study the neural basis of natural behaviors in a wide range of animals without the need for genetic tools or specialized equipment.

Poster Session 1 | Poster Wall 106 | Label: PS1.106

Category: Methods and education

Transient and persistent fear states in Drosophila melanogaster

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In response to threatening stimuli, animals exhibit one of three defensive behaviors: freezing, fleeing, or fighting. Remarkably, across a wide range of taxonomic groups, with unique morphologies and distinct environmental niches, these strategies are consistently employed in a context-dependent manner. This convergence highlights their significant evolutionary value. The fruit fly, with its rich defensive behaviors and tools for dissecting neuronal circuits, is an invaluable model to explore the basis of these responses.

We developed a high-throughput behavioral assay – from experimental setup to analysis pipeline – to investigate the responses of fruit flies to repeated, inescapable visual looming stimuli, simulating a predator's recurrent attacks after unsuccessful attempts to catch its prey. This assay enables us to differentiate two phases of defensive responses: a startle response that coincides with stimulus presentation and exhibits a transient profile, and subsequent fear states that can persist for extended periods, over minutes, including sustained freezing and running.

We tested the role of various loom-responsive visual projection neurons (VPNs). Freezing responses were largely mediated by LC6 neurons, whose activity is necessary and sufficient to drive freezing. In addition, LPLC2 neurons were required for normal freezing responses to looming stimuli, but unlike LC6 neurons their silencing also decreased escape responses. Interestingly, activation of LPLC2 neurons reliably drove escapes, only weakly eliciting freezing after multiple rounds of stimulation. Finally, we found various VPNs (LC4, LC16 and LPLC1) specifically implicated in escape responses. In summary, sustained freezing and escape responses are mediated by several, largely non-overlapping VPNs, being LPLC2, that directly projects to descending neurons known to drive escape (giant fiber) and freezing (DNp09) responses, implicated in both.

Poster Session 1 | Poster Wall 107 | Label: PS1.107

Category: Methods and education

Longitudinal functional brain imaging in juvenile zebrafish and adult danionella

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Larval zebrafish (Danio rerio) are an attractive model in systems neuroscience due to their small size and optical accessibility, thereby enabling non-invasive whole-brain imaging in live animals. However, higher cognitive functions (e.g., associative learning) only become robust later in development (from 2 to 3 weeks post-fertilization), wherein minimally restraining the fish becomes more difficult. This challenge is also true for live imaging of the emerging fish model, adult danionella (Danionella cerebrum). Hence, we developed a technique combining agarose and an ultraviolet-curable polymer to restrain juvenile zebrafish and adult danionella for multi-day non-invasive imaging. With this method, tethered fish can breathe actively (i.e., without intubation) as well as move their eyes and tail freely during experiments. We demonstrate the feasibility of this technique by monitoring the behavioral responses of tethered fish upon stimulus presentation while recording neuronal activity in different brain regions using two-photon microscopy.

Poster Session 1 | Poster Wall 108 | Label: PS1.108

Category: Methods and education

Measuring raptor prey-capture behavior during natural flight

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Birds of prey use a wide range of behavioral strategies when capturing airborne prey, but while many studies have tracked bird behavior during flight using a variety of approaches, a method for capturing flights in combination with eye motion with high resolution has not been developed. Here, we present an analysis of Harris's Hawks (Parabuteo unicinctus) prey capture behavior using high-resolution imaging from a light-weight, tether-free camera and tracking system that could be mounted on the bird's head and body. We first trained Harris's Hawks using a combination of falconry techniques and mock equipment with increasing dimensions and weights, enabling the birds to perform flights largely unaffected by the recording equipment. To maintain image stability throughout the entire flight, we designed and adapted a specifically customized hood that offered a firm hold for data recording accuracy, while ensuring a comfortable and unobtrusive fit for the bird. We then used this system to track prey capture behavior, ranging from simple flights between two stationary perches to capturing airborne prey targets. The resulting high-resolution images allowed us to measure eye and head rotations during flight, prey tracking and capture, and landing behaviors.

Poster Session 1 | Poster Wall 109 | Label: PS1.109

Category: Methods and education

From the animal's point view: A head-mounted 4pi camera approach for accurate tracking of position, attitude and behavior in natural environments

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Precise quantification of behavior in natural environments is challenging, but essential for neuroethologic research. While in controlled lab environments external cameras have been used for sub-millimeter-accurate tracking of animal position and orientation, more complex behaviors require complex environments with which these methods are incompatible. In natural environments, long distance tracking has been achieved with GPS and general classification of orientations with inertia measurement units (IMU). However, GPS accuracy is limited and IMU sensor fusion is too slow for highly dynamic situations. To overcome these limitations, we developed a light-weight tetherless, omnidirectional (4π) camera combined with an IMU, designed to be mounted on an animal freely moving in the wild. We calibrated this camera and developed custom visual odometry and Kalman filter software to infer position and orientation solely based on the videography and acceleration data recordings from the sensors on the animal. This allowed reconstruction of both trajectory and behavior in a drone-scanned and digitally reconstructed environment of more than 10,000 m². We applied this approach to observe eye movements in freely flying Harris's hawks during prey capture tasks in an open field. We reconstructed the animals' trajectories with spatial accuracies of

Poster Session 1 | Poster Wall 110 | Label: PS1.110

Category: Methods and education

Recording electrical activity from the brain of behaving octopus

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Octopuses, which are among the most intelligent invertebrates, have no skeleton and eight flexible arms whose sensory and motor activities are at once autonomous and coordinated by a complex central nervous system. The octopus brain contains a very large number of neurons, organized into numerous distinct lobes, the functions of which have been proposed based largely on the results of lesioning experiments.In other species, linking brain activity to behavior is done by implanting electrodes and directly correlating electrical activity with observed animal behavior. However, because the octopus lacks any hard structure to which recording equipment can be anchored, and because it uses its eight flexible arms to remove any foreign object attached to the outside of its body, in vivo recording of electrical activity from untethered, behaving octopuses has thus far not been possible. Here, we describe a novel technique for inserting a portable data logger into the octopus and implanting electrodes into the vertical lobe system, such that brain activity can be recorded for up to 12 h from unanesthetized, untethered octopuses and can be synchronized with simultaneous video recordings of behavior. In the brain activity, we identified several distinct patterns that appeared consistently in all animals. While some resemble activity patterns in mammalian neural tissue, others, such as episodes of 2 Hz, large amplitude oscillations, have not been reported. By providing an experimental platform for recording brain activity in behaving octopuses, our study is a critical step toward understanding how the brain controls behavior in these remarkable animals.

Poster Session 1 | Poster Wall 111 | Label: PS1.111

Category: Motor systems, sensorimotor integration, and behavior

Sensorimotor calibration in optic flow processing circuits

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Exploratory animals structure their behavior to maximize gaze stability, thereby facilitating the acquisition of visual and spatial information while minimizing retinal slip. Such gaze control depends on multisensory integration, but the circuit mechanisms underlying precise multimodal calibration during locomotion remain unclear. Partially, this is because of the highly distributed nature of sensorimotor circuits, making it challenging to identify circuits involved in goal-directed gaze control. Here we take advantage of Drosophila melanogaster's compact Central Nervous System to examine multimodal calibration during exploratory walking. We tested whether flies maintain gaze stability under visual perturbations by immersing them in a virtual world and subjected them to constant rotations of this world. Flies adjusted their velocity to preserve gaze stability, underscoring visuomotor recalibration. To begin to understand the neural underpinnings of such recalibration, we adapted this parading to head-fixed flies walking on a spherical treadmill and recorded neural activity in simultaneous from a population of genetically identified neurons involved in gaze control. The GABAergic bIPS cells receive multimodal information from integrative brain regions and the VNC (the insect analogue of the spinal cord), providing an anatomical substrate for calibration. Recordings from bIPS in walking flies showed that they congruently combine retinal and extra-retinal signals. Moreover, this congruent multimodal combination sharpens the neuron's sensitivity to the body's translation and rotation. Ongoing experiments are testing the activity of bIPS under visual perturbations. Together, our data underscores the properties of an integrative inhibitory hub involved in steering during locomotion. Future work leveraging the EM connectomics datasets will test the mechanisms by which bIPS combine and calibrates multimodal information for gaze control.

Poster Session 1 | Poster Wall 112 | Label: PS1.112

Category: Motor systems, sensorimotor integration, and behavior

Electrophysiological differences between multifunctional and behaviorally specialized turtle spinal neurons involved in swimming, scratching, and flexion reflex

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The adult turtle spinal cord can generate multiple kinds of limb movements, including swimming, three forms of scratching, and limb withdrawal (flexion reflex), even without brain input and sensory feedback. There are many multifunctional spinal neurons, activated during multiple motor patterns, as well as some behaviorally specialized neurons, activated during only one. How do multifunctional and behaviorally specialized neurons each contribute to motor output? We analyzed in vivo intracellular recordings to assess electrophysiological differences between multifunctional and specialized neurons. Neurons tended to spike in the same phase of the hip-flexor activity cycle during swimming and scratching, though one cell preferred opposite phases for the two behaviors. There were more highly rhythmic multifunctional neurons than scratch-specialized and flexion reflex-selective neurons, with one group active during the hip flexor-on phase and another during the hip flexor-off phase. Thus, hip flexor-extensor alternation may be generated by a subset of multifunctional spinal neurons during both swimming and scratching. Scratch-specialized neurons and flexion reflex-selective neurons may instead trigger their respective motor patterns, by biasing activity of some multifunctional neurons. In phase-averaged membrane potentials of multifunctional neurons, trough phases were more highly correlated between swimming and scratching than peak phases, suggesting that rhythmic inhibition plays a greater role than rhythmic excitation. We also found the first turtle swim-specialized neuron: tonically excited during swimming, but inactive during scratching and flexion reflex. It displayed an excitatory postsynaptic potential following each swim stimulation pulse and thus may be an intermediary between reticulospinal axons and the swimming CPG they activate.

Poster Session 1 | Poster Wall 113 | Label: PS1.113

Category: Motor systems, sensorimotor integration, and behavior

Anatomical exploration of motoneurons driving woodpecker drumming behavior

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Motoneuron structure and motor pool anatomy can offer insights into how organisms produce complex behaviors and/or specialized reflexes. In some cases, motoneurons show distinct specialization to facilitate these movements, whether in size, branching, or endocrine sensitivity. We aimed to quantify and describe the motoneuronal architecture which underlies the production of woodpecker drumming. The drum is a territorial acoustic signal that female and male downy woodpeckers create by hammering their beak on hard substrates. Drums vary in a few different signal components, at least two of which (drum speed and drum length) indicate some aspect of individual quality or territorial strength. To produce this display, we expect the woodpecker motor system to be modified to support extreme head and neck movements. Here, we take a first look at the motoneuron architecture involved in drumming. We used retrograde tract tracing to fluorescently label motoneurons that innervate the primary drumming muscles in the neck: longus colli ventralis and longus colli dorsalis. We measured soma size and the extent of motor pools for each neck muscle, as well for the scapulohumeralis (a non-drumming shoulder muscle control). We find that cell body size and motor pool extent are indistinguishable for the two neck muscles, and the control shoulder muscle motoneurons have larger cell bodies. When compared to a non-drumming control species (the house sparrow), we find no species specificity in motoneuron size or extent of the motor pool. Together, our findings suggest that specialization for drumming, if it exists, does not occur in the cell body size of motoneurons. Additionally, our results are the first step to mapping a complete drum motor circuit, an avenue of future work for this system.

Poster Session 1 | Poster Wall 114 | Label: PS1.114

Category: Motor systems, sensorimotor integration, and behavior

Flies tune the sensitivity of their multifunctional gyroscope

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Locomotion requires navigating unpredictable and complex environments, demanding both stability and maneuverability within short timeframes. This is particularly important for flying insects, and the true flies (Diptera) stand out among this group for their impressive flight capabilities. Flies' aerial abilities are partially attributed to halteres, tiny club-shaped structures that evolved from the hindwings and play a crucial role in flight control. Halteres oscillate during flight, in antiphase with the wings, providing rhythmic input to the wing steering system via arrays of embedded mechanosensors called campaniform sensilla. These sensor arrays convey timing information to the wing steering muscles, but linking haltere sensor location to sensor activity and the functional organization of the wing steering system remains a central challenge. Here, we use in vivo calcium imaging during tethered flight to obtain population-level recordings of the haltere sensory afferents in specific fields of sensilla. We find that haltere feedback is continuously modulated by visual stimuli to stabilize flight. Additionally, this feedback is present during saccades and help flies actively maneuver. We also find that the haltere's multifaceted role arises from the steering muscles of the haltere itself, regulating haltere stroke amplitude to modulate campaniform activity. Taken together, our results underscore the crucial role of biomechanics in regulating the dynamic range of sensors, and provide insight into how the sensory and motor systems of flies coevolved.

Poster Session 1 | Poster Wall 115 | Label: PS1.115

Category: Motor systems, sensorimotor integration, and behavior

Behavioral and neural correlates of a supernormal song stimulus in Nightingales

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Coined by Niko Tinbergen, a 'supernormal stimulus' indicates an exaggerated version of a natural stimulus eliciting an enhanced behavioral response. Supernormal visual stimuli have been behaviorally investigated in several animal species. However, other sensory modalities and the neural representations of supernormal stimuli are unknown. Here, we identified a supernormal song stimulus for nightingales and explored the neural correlates that might explain the enhanced behavioral response. Nightingales, songbirds with a repertoire of up to 200 different songs, perform sophisticated night-long song duels against conspecifics. During these duels, they perform song-matching, copying repertoire-shared songs of their rivals. To test whether the bird's own songs (BOS) act a as supernormal song stimulus, we conducted field experiments combined with accelerometry recordings, exposing wild nightingales to playbacks simulating different opponents. BOS playbacks elicited enhanced behavioral responses compared to conspecific's playback increasing the movement activity of singing nightingales, indicative of an elevated state of arousal of the birds. Using a deep learning approach, we found that nightingales tripled the song matches and even anticipated song sequences of BOS playbacks compared to conspecific playbacks, revealing BOS as a supernormal song stimulus redirecting preexisting singing patterns. Next, we investigated the neural correlates of song stimuli by performing electrophysiological recordings in hand-raised and song-tutored nightingales. Neurons in the bird's premotor area HVC selectively responded to the presentation of songs, with precisely time-locked responses to playbacks of the bird's own songs. Taken together, these results demonstrate how nightingales' own songs act as supernormal stimuli, driving exaggerated behavioral responses in wild animals and evoking precise auditory integration in a premotor circuit in the bird's brain.

Poster Session 1 | Poster Wall 116 | Label: PS1.116

Category: Motor systems, sensorimotor integration, and behavior

Descending control and regulation of spontaneous flight turns in Drosophila

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The clumped distribution of resources has influenced the pattern of foraging behavior since the origins of locomotion, selecting for a common search motif in which straight movements through resource-poor regions alternate with zig-zag exploration in resource-rich domains. For example, during local search, flying flies spontaneously execute rapid flight turns called body saccades, but suppress these maneuvers during long-distance dispersal or when surging upstream towards an attractive odor. Here, we describe the key cellular components of a neural network in flies that generate spontaneous turns as well as a specialized pair of neurons that inhibits the network and suppresses turning. Using 2-photon imaging, optogenetic activation, and genetic ablation, we show that only four descending neurons appear sufficient to generate the descending commands to execute flight saccades. The network is organized into two functional units—one for right turns and one for left—with each unit consisting of an excitatory (DNae014) and inhibitory (DNb01) neuron that project to the flight motor neuropil within the ventral nerve cord. Using resources from recently published connectomes of the fly, we identified a pair of large, distinct interneurons (VES041) that forms inhibitory connections to all four saccade command neurons and created specific genetic driver lines for this cell. As predicted by its connectivity, activation of VES041 strongly suppresses saccades, suggesting it promotes straight flight to regulate the transition between local search and long-distance dispersal, Using the connectome, we have also identified a number of additional neurons that are well suited to regulate brief bouts of turns that flies exhibit during the final stages of odor localization. These results thus identify the key elements of a network that may play a crucial role in foraging ecology. Comparative evidence suggests this network may represent an ancient core circuit within the insect brain.

Poster Session 1 | Poster Wall 117 | Label: PS1.117

Category: Motor systems, sensorimotor integration, and behavior

Hummingbird hawkmoths display lateralised visuo-motor control of the proboscis during pattern inspection

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Directional biases in behaviour and limb use are present in a wide range of animals – from primates to insects – referred to as ‘handedness’ or ‘lateralisation’. However, despite the prevalence of such motor properties, the advantage they confer is still not entirely clear. Proposed benefits of lateralisation have largely been considered in social contexts, useful for coordinating behaviours with conspecifics (Niven and Frasnelli (2018) Prog. Brain. Res. 238: 3-31). For animals, particularly insects, in non-social contexts, it has been argued that a directional motor specialization may confer advantages in the performance of specific actions or motor control tasks (Niven & Bell (2018) Trends Ecol. Evol. 33(7)), but remains to be quantified and tested in a continuous fine-tuned motor task (see Kannegieser et al. (2024) PNAS 121(6)).

While foraging, the hummingbird hawkmoth (Macroglossum stellatarum) uses visual pattern features to continuously guide their proboscis to a flower’s central nectary. Here, we use pose estimation (DeepLabCut) to track free-flying hawkmoths while inspecting such patterns on 2D discs. By analyzing the proboscis contact positions on the disc relative to the head axis (proboscis base – head centre), we show a continuous range of lateralisation in proboscis placement across individuals, but no population-level bias. Additionally, we find that some moths are less consistent in their lateralisation than others, with bias direction and strength varying among flight bouts. Ongoing experiments aim to reveal how stable an individual’s bias is over time, if flower pattern features influence biases, and whether the lateralisation in proboscis placement is mediated by a potential eye dominance. Moving forward, we will also measure the latency to find the nectary (pattern centre) once contact is established between the proboscis and the pattern, aiming to discern how lateralisation is linked to visuo-motor performance in a foraging task.

Poster Session 1 | Poster Wall 118 | Label: PS1.118

Category: Motor systems, sensorimotor integration, and behavior

Obstacle avoidance in Locusta migratoria: furthering understanding through combined techniques in electrophysiology, behavioral recording, and stimulus generation

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Averting collisions with the environment, crashes with conspecifics, and capture by predators—these are all behaviors within the repertoire of locusts, enabled by visuomotor pathways incorporating well-studied visual interneurons such as the Lobula Giant Movement Detector (LGMD) and Descending Contralateral Movement Detector (DCMD). The behaviors controlled by these pathways are also well-studied in terms of relationships between stimuli, wingbeat kinematics, and ensuing flight trajectories. However, the vast majority of the work currently present in the literature has studied both the visual and motor aspects of locust collision avoidance using open-loop methods, thus leaving many unanswered questions regarding how the animal's flight kinematics modify the stimulus, and the locust's continued response to it, over time. Moreover, simultaneous studies of visual interneuron output and flight behavior are limited. We developed a set of novel apparatus and methods to address these gaps, namely: a method for real-time measurement of wingbeat kinematics correlated with obstacle-avoiding turns, and a method for minimally-intrusive recording of visual interneuron output from largely intact animals in tethered flight. These methods lay the foundation for examination of obstacle avoidance behaviors in both open- and closed-loop contexts, and further our understanding of interactions between visual and motor systems in locust flight. Such investigations will improve our understanding of a classic model for visuomotor behavior, and further its use as a basis for artificial collision detectors based on computational modelling by elucidating how visual outputs are interpreted.

Poster Session 1 | Poster Wall 119 | Label: PS1.119

Category: Motor systems, sensorimotor integration, and behavior

Recovery of locomotion after injury: Expression of voltage-gated ion channels and biogenic-amine receptors in the leech nerve cord after removal of descending inputs

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It has been demonstrated previously that after transection between the brain and segmental ganglia leeches are refractory to crawling. However, after 10-14 days they recover their ability to crawl, in part, through the emergence of a new “lead” ganglion directly below the transection site. Subsequently, it has been shown that remodeled peripheral proprioceptors likely play a key role in this recovery. We have hypothesized that neurons of the lead ganglion also undergo changes in excitability and receptiveness to modulation that may underlie crawl recovery. To begin to test this hypothesis, we measured in the anterior and posterior ganglia of transected and sham-control animals ion channel and amine receptor mRNAs. The nerve cords of animals were transected between ganglion M2 and M3, and all ganglia were then harvested for analysis. qPCR results indicated a substantial decrease in expression of most biogenic amine receptors in the M3 ganglia of acutely (3 days post-transection) injured animals. Conversely, we found distinct changes in amine receptors, including serotonin and dopamine, in chronically injured animals that had recovered crawling. Specifically, some receptor mRNAs were significantly elevated in the lead ganglia and decreased in the ganglia anterior to the transection site. In addition, we found an increase in mRNAs encoding voltage-gated K+ channels in ganglion M3. Lastly, we detected substantial changes in Actin expression in these same lead ganglia, which may indicate morphological reorganization commensurate with remodeling of central arbors belonging to identified proprioceptors. Our results suggest that neuroplasticity, associated with crawl-related compensation, likely includes changes in channel and receptor expression that influence the firing properties of neurons in the lead ganglion. Future experiments will take a cell-specific approach to determine in which cell types these changes may be most prominent to influence locomotor recovery.

Poster Session 1 | Poster Wall 120 | Label: PS1.120

Category: Motor systems, sensorimotor integration, and behavior

Twitch kinetics set neuromuscular limits on the performance of territorial drums in downy woodpeckers (Dryobates pubescens)

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Many animals court mates and compete with rivals through showy and elaborate displays, but understanding performance differences amongst individuals and species has been challenging. For example, many speculate that systems of motor control may act at different locations along the neuromuscular apparatus to set major limits in display performance, yet few studies rigorously test this notion. We do so here by exploring how skeletal muscle performance might constrain different elements of the elaborate drum displays that male downy woodpeckers (Dryobates pubescens) use to compete for breeding territories. Specifically, we apply in situ twitch challenges to the neck muscle that powers drumming (longus colli ventralis, or LCv) to assess how performance limits in the musculature determine variation in display speed (beats sec -1) and length (total beats). We found that twitch times appear to limit drum speed, given that stimulation frequencies that mimic drums faster than those performed in nature cause fusion in the muscle. However, the LCv appeared resistant to any fatigue accumulation when presented with drums twice the length of those performed in nature. Further evidence from these in situ twitch assays suggests they may also set informative performance limits for species recognition of drums as well. Muscle performance therefore likely imposes differential constraints of discrete components of the drum, a possibility that has received very little attention previously.

Poster Session 1 | Poster Wall 121 | Label: PS1.121

Category: Motor systems, sensorimotor integration, and behavior

Application of System Identification to Model Bat Echolocation Parameter Control During Prey Tracking

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During predation, big brown bats (Eptesicus fuscus) produce frequency modulated (FM) echolocation sweeps that exhibit rapid changes in duration, interval, and spectral features as they search for, track, and intercept prey (Schnitzler and Kalko, 2001; Simmons et al., 1979). Modeling how insectivorous bats modulate their echolocation calls with respect to the motion of their prey has been crucial for identifying the importance of target motion prediction and inter-call timing optimization during tracking (Erwin et. al 2001; Luo et. al 2017; Moss et al., 2004; Salles et. al 2020).

In this study, we apply an approach from control theory, known as system identification, to quantify the relationship between echolocation parameters and target distance. The bats are systematically exposed to prey targets that move back-and-forth over a range of oscillation frequencies. A broad range of motion frequencies is used to understand the way bats process different movement features (e.g., slow, steady motion or rapidly changing motion) through their echolocation parameters. We use these motions to create empirical response functions which map changes in target motion to changes in echolocation behavior.

To gain further intuition into the mapping between target motion and echolocation parameters, numerical transfer function models, such as delayed high-, low-, and band- pass filters, are fit to the observed response functions. These transfer functions provide insight into the aspects of target motion the bat prioritizes and quantify the processing delays that may exist during active sensing. Thus far, we have demonstrated that pulse duration and pulse interval modulation may have different roles in target motion processing and have quantitatively modeled the processing delay in the implementation of these echolocation call parameters.

Poster Session 1 | Poster Wall 122 | Label: PS1.122

Category: Motor systems, sensorimotor integration, and behavior

Signal transmission among leech ganglia

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Leeches display a robust motor behavior that allows them to move on solid surfaces, through elongation and contraction waves along the longitudinal axis. The neuronal network that controls the crawling behavior can be studied at the level of the isolated nervous system (crawling), where recordings of identified motoneurons show a motor pattern compatible with the behavior. Isolated nerve cords or single ganglia, treated with dopamine, exhibit rhythmic activation of the motoneurons responsible for elongation and contraction with due timing.

To study signal transmission among ganglia we analyzed the motor pattern elicited in chains of three ganglia, disconnected from the cord and from the periphery. The three interconnected ganglia show coordinated rhythmic motor activity, indicating that they exchange signals that grant a basic correlated rhythmic activity (Kearney et al, 2022). To further analyze inter-ganglionic signals we used chains of three ganglia that were chemically compartmentalized, where only the anterior or the posterior ganglion were treated with dopamine while the rest of the chain was in normal saline. We observed that the stimulated ganglion exhibited a crawling pattern as that observed in isolated ganglia. Adjacent anterior or posterior ganglia displayed rhythmic activity that could be classified into “bursting” (high frequency separated by silent phases) or “modulated” (high frequency separated by low frequency phases), tuned to the stimulated ganglion. The analysis of the data shows that rhythmic activity is transmitted among ganglia in both directions. These signals are not sufficient to establish coordinated crawling in the chain but may contribute to its establishment.

Poster Session 1 | Poster Wall 123 | Label: PS1.123

Category: Motor systems, sensorimotor integration, and behavior

The neurobiology of visually-guided ambush behaviour in South American horned frog

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Predatory behaviors are guided by sensorimotor systems and are adapted for diverse environments and hunting strategies. Frogs provide an important evolutionary model for studying relationships between sensory systems and lifestyle. Early neuroethological studies on common toads revealed stereotypic predatory behavior triggered by visual, worm-like moving stimuli. Subsequent studies on other actively hunting frog species identified similar action patterns and stimulus preferences. Neuronal responses recorded in the visual center, the optic tectum, revealed specific neurons with preference to worm-like moving stimuli.

In contrast, South American horned frogs (Genus Ceratophrys) are sedentary ambush predators that attack most moving prey entering their immediate vicinity. Field studies show they typically remain burrowed, preying opportunistically on insects, rodents, reptiles, birds, and other frogs; in some instances when ambushing other frogs, they use their hind legs as visual lures. This research aims to study neuronal responses underlying these unique predatory behaviors, focusing on behavioral preferences, physiological responses to different visual stimuli, and retinotopic organization in the optic tectum.

Behavioral experiments revealed relatively non-specific tuning to a range of moving stimuli of varying sizes, with a slight preference for large, familiar moving objects. In vivo extracellular recordings identified distinct neuronal classes preferring discrete stimulus orientations, with approximately 40% preferring worm-like stimuli. We found that specific neurons excited by worm-like movement also responded to frog-generated luring movements. Within the Ceratophrys tectum, the retinotopic map has distinct elaborations for superior, nasal fields, in congruence with the forward position of the eyes within this species.

Poster Session 1 | Poster Wall 124 | Label: PS1.124

Category: Motor systems, sensorimotor integration, and behavior

Adaptive flight strategies for challenging light environments in the nocturnal hawkmoth Deilephila elpenor

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Flying insects use visual information to control and stabilize their flight. In nature, this information is highly dynamic, and thus presents a challenge for insect brains to extract reliable and relevant information from it. And yet, many animals master this challenge on a daily—and nightly—basis. To understand this ability, it requires studying both sensory processing and behaviour, which act in concert: as senses guide an animal's movements, the movements in turn shape the sensory input. In flying insects for example, alterations in flight speed and distance to objects shape the spatiotemporal frequency composition of their visual input. Shaping visual inputs by adaptive movement can be particularly important in challenging light environments – in very dim light, or when light levels change suddenly.

Here, using the nocturnal hawkmoth Deilephila elpenor, we studied how flight features are adjusted to support visual acquisition at different natural light intensities. To record the moths' flights, we used a large-scale motion capture system (12x12x6m arena with 38 cameras), where animals could choose their flight strategies flexibly and use their full repertoire of flight manoeuvres. Moreover, ambient light intensities were matched to three natural conditions within the moth's visual activity range (starlight, moonlight, and twilight). We discuss how the visual environment impacts the animals' flight performance, measured as translational velocity, flight height off the ground, and distance to surrounding structures. We set this in relation to the spatial frequency composition of the available visual information and known processing properties of the brain.

Poster Session 1 | Poster Wall 125 | Label: PS1.125

Category: Motor systems, sensorimotor integration, and behavior

Descending control of flight saccades in Drosophila

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Flies perform rapid turns termed saccades to change direction during flight. Evasive turns can e.g. be elicited by looming stimuli mimicking an approaching object such as a potential predator. Whereas projection neurons of the optic lobes responsive to looming stimuli have been well described, how this information is transmitted to the motor system to elicit a saccade, is not well understood. Here, I describe a descending neuron, DNp03, which receives direct input from looming-sensitive visual projection neurons and projects to wing motor areas within the ventral nerve cord. Whole-cell patch-clamp recordings from this neuron during head-fixed flight confirm that DNp03 is responsive to looming stimuli on the side ipsilateral to its dendrites. In addition, activity of this neuron is state-dependent as looming stimuli only elicit spikes during flight and not rest. A comparison between neuronal activity and turning response, measured as change in left minus right wing stroke amplitude, revealed a correlation between the spiking activity of DNp03 and the strength and timing of the behavioral response. However, this correlation is not very strong suggesting the involvement of other descending neurons in controlling saccadic turns. Nevertheless, DNp03 is a prime candidate for controlling evasive saccades during flight. In addition, its activity is also modulated during spontaneous saccades that are not linked to the presentation of looming stimuli, suggesting that the pathways for controlling both types of saccades are not separate. To study the role of this and other descending neurons during naturalistic behavior, we have developed a free flight setup that allows us to optogenetically activate DNs during free flight. This work provides an entry point into understanding how sensory information is transmitted to the motor system to control an important behavior of the fly.

Poster Session 1 | Poster Wall 126 | Label: PS1.126

Category: Motor systems, sensorimotor integration, and behavior

Effects of sublethal dose of pesticides on visually guided behaviour of European Honeybees (Apis mellifera)

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The European honeybees (Apis mellifera) are abundant and effective natural pollinators contributing enormously to mass production of agricultural crops through extensive foraging behaviours. This group, like most insects, possesses visual systems that are highly sensitive to motion, and efficiently enhance navigation within their complex environment with high precision. They rely on motion of visual elements (optic flow) within their environment to guide sophisticated behaviours and communication. Optic flow stimulates an innate response known as the optomotor response, which enables the bees to orient and maintain a straight course during flight. Agricultural chemicals and other stressors in the environment negatively impact the ability of honeybees to engage in these economically relevant behaviours. These compounds are currently and persistently being used to treat seeds of crops, despite their negative effect on non-target organisms like honeybees – and include the common neonicotinoids and the novel sulfoximines. Previous studies showed that these compounds impaired walking tracks associated with optomotor responses. This study is investigating the effects of two pesticides, imidacloprid and sulfoxaflor, on bee optomotor response in a newly designed 360° virtual reality arena. Initial analysis indicates effects on orientation behaviour and further analysis will examine specific effects on walking tracks and head orientation to stimuli that evoke robust behaviour in control bees.

Poster Session 1 | Poster Wall 127 | Label: PS1.127

Category: Motor systems, sensorimotor integration, and behavior

Role of Behavioral Rules and Feedback Cues in Shaping Social Interactions

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Social interactions are shaped by the behavioral decisions and resulting feedback of interacting organisms. Interactions with different partners can vary, but the relative contribution of an individual's actions and the partner's feedback to this variation is often unclear. We dissect the role of behavioral rules and feedback in shaping social interactions using Drosophila melanogaster courtship as a model. During courtship, males dynamically process sensory feedback from the female to pattern their courtship song. The male is typically behind the female and a receptive female slows down in response to song. Interestingly, Drosophila males also court other males and these interactions look remarkably different: the target male often sings back or is aggressive leading to head-to-head interactions. These target-specific interactions are also accompanied by differences in courtship song decisions. We therefore asked whether the males employ different sensorimotor strategies for patterning his courtship song towards different targets. Using computational modeling, we found that males use the same set of sensorimotor rules to sing towards both sexes since models with target-specific rules do not outperform a target-agnostic model. Conversely, the target-specific song patterning arises from flexible selection of these rules based on the target-specific feedback. We further investigated the unique head-to-head context during male-directed interactions, discovering that the male target's turning response induced by courtship song perception initiates this context. The internal arousal state of the flies shape this context through central brain neurons P1a, as demonstrated by our optogenetic activation experiments. In summary, our research shows that while the target behavioral feedback drives the interactions in target-specific ways, a flexible choice of sensorimotor rules allows the organism to reach appropriate behavioral decisions during social interactions.

Poster Session 1 | Poster Wall 128 | Label: PS1.128

Category: Motor systems, sensorimotor integration, and behavior

Action, valence, dopamine- Drosophila as a study case

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Darwin´s 1872 book on the relationship between emotions and behavior (Darwin 1872) sparked a controversy continuing to this day. He suggested that there can be mutual causation between these two: not only can a particular emotional state engage a corresponding expressive behavior, but, conversely, we can adopt the emotional state corresponding to the behavior we engage in.

To see whether a neurobiologically tractable study case to investigate these processes can be established in the fruit fly Drosophila melanogaster we focused on basic behaviors and emotions, namely moving forward or backward and feeling 'good' or 'bad'. We induce forward locomotion by activating the 'Bolt' neurons (Bidaye et al. 2020) and find that odors presented during such forward locomotion can acquire positive valence. In turn, inducing backward locomotion by activating the 'moonwalker neurons' (Bidaye et al. 2014) can establish negative valence.

Through a combination of behavioral analyses, optogenetics, pharmacology, connectomics, neurophysiology and modelling, we investigate the punishing effect of activating the moonwalker neurons in detail, with a focus on the pathways from the mushroom body towards the motor periphery, and the role of movement and of the dopaminergic reinforcement system in this paradigm. A normative model inspired by the uncovered processes suggests that they can maintain successful learned avoidance, shedding new light on what is known in experimental psychology as the 'avoidance paradox'.

Poster Session 1 | Poster Wall 129 | Label: PS1.129

Category: Motor systems, sensorimotor integration, and behavior

Spatiotemporal Pulsatile Floating Pattern Analysis for Cyborg Jellyfish Control

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Jellyfish (Aurelia aurita medusae), with their extremely simple body and neural structures, can adaptively float in complex and dynamically fluctuating water, demonstrating one of the highest energetic efficiencies among aquatic animals. Although previous studies have attempted to understand the mechanism of floating behavior based on hydrodynamics and biomechanics, the neural control mechanism of the jellyfish remains unknown. Elucidating this mechanism will also contribute to the development of cyborg jellyfish that can be used for marine surveillance and ocean cleanup. Here, we developed a three-dimensional measurement system that enables comprehensive analysis of the pulsatile floating locomotion of the jellyfish and a setup for their muscle electrical stimulation that enables locomotion control. Experimental results using the developed intervention system revealed microscopic spatiotemporal patterns of soft body deformation in response to muscle electrostimulation for the floating jellyfish. A further detailed analysis of the relation between the micro- and macroscopic behaviors, such as floating direction and speed, would improve the navigational capability of jellyfish cyborgs.

Poster Session 1 | Poster Wall 130 | Label: PS1.130

Category: Motor systems, sensorimotor integration, and behavior

Two-Photon imaging in freely singing birds

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Song of most bird species is a seasonal behaviour and depends on gonadal hormones, testosterone and estrogen. These hormones affect the singing activity and song patterns, the latter being controlled by the so-called song control system. The study of the neural mechanisms underlying the song pattern shall inform about the coding of sequential motor actions that in sum represent the song. Since the song patterns can be modified by testosterone treatment, we used miniaturized head-mounted 2-Photon microscopy (MINI2P) within the same animal to determine the neural pattern of sequential singing. MINI2P has enabled imaging of neuronal activity in freely moving mice. However, 2-Photon imaging in freely moving birds remained challenge, since diurnal birds do not active in darkness while the photon detectors of 2-Photon microscopes are extremely light-sensitive. Here we synchronized environmental light with the scanner, enabling long-term functional imaging of neuronal activity in freely singing zebra finches in lit environment.

Poster Session 1 | Poster Wall 131 | Label: PS1.131

Category: Motor systems, sensorimotor integration, and behavior

Descending pathways that control the initiation, speed, and halting of locomotion in Drosophila

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The brain controls high-level aspects of movement including the initiation, speed, and halting of locomotion. These control signals engage motor circuits in the spinal cord (in vertebrates) or ventral nerve cord (in invertebrates) via descending pathways. How these pathways are organized and recruited, and how they engage the motor circuits remains poorly understood. Using an optogenetic activation screen, we identify specific neurons in the Drosophila brain that control the initiation/speed and halting of locomotion in freely walking animals. Selective activation of the speed neurons increases walking speed as a function of activation intensity. Selective activation of the halting neurons halts locomotion in a naturalistic manner. Using single-cell patch clamp recordings, we find that the halting neurons are activated by mechanosensory input from the antennae, suggesting that the halting pathway can be recruited by external mechanical stimuli. Using connectomes of the fly brain, we comprehensively analyze the inputs and outputs of the speed and halting neurons and identify descending neurons that carry their signals to the motor circuits that control the legs. The halting neurons directly target a specific descending neuron, whereas the speed neurons indirectly target multiple descending pathways. In ongoing experiments, we combine two-photon calcium imaging with automated behavioral analysis and leg tracking to elucidate how these pathways are recruited to mediate adaptive locomotion depending on the behavioral context.

Poster Session 1 | Poster Wall 132 | Label: PS1.132

Category: Motor systems, sensorimotor integration, and behavior

Consistency vs. flexibility of motor circuits: convergent neuropeptide comodulation pulls the stomatogastric pyloric circuit towards activity patterns that are independent of modulator identity

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Neural circuits are under the influence of multiple modulators, and comodulation is thought to increase flexibility by expanding the repertoire of possible circuit states. However, different modulators often overlap in their targets and therefore have convergent and occluding effects. Here we propose an additional role for comodulation: that convergent effects of comodulators can increase similarity of circuit output. In the pyloric circuit of the crustacean stomatogastric ganglion, excitatory neuropeptides converge to activate the same ionic current. At the circuit level, however, different neuropeptides act on different subsets of neurons. If convergence at the cellular level is predominantly additive, increasing numbers of comodulators could eventually activate all circuit neurons in the same way, rendering the identity of the modulators unimportant.

To test whether increasing numbers of convergent modulators produce progressively similar circuit outputs, we applied peptide modulators as a singlet, doublet, and triplet (A, A+B, A+B+C), then washed and applied a second set (D, D+E, D+E+F). We quantified activity attributes (cycle freq, burst phases, intraburst spike# and freq) under each condition and calculated the distance between any two circuit outputs in the multidimensional space of all attributes. By comparing the differences among the singlet (A vs. D), doublet (A+B vs. D+E), and triplet (A+B+C vs. D+E+F) applications we found that circuit outputs became increasingly similar from singlets to triplets, independent of the identity of the modulators. Mathematical analysis shows that increase in similarity is robust to variability of dose-response curves and is enhanced by sublinear summation of modulator effects. Our results suggest that comodulation can either pull circuits toward a common output activity or push it toward different output patterns. The actual circuit output then depends on the balance of converging and diverging modulatory actions.

Poster Session 1 | Poster Wall 133 | Label: PS1.133

Category: Motor systems, sensorimotor integration, and behavior

Avoid or Attack? Context-dependent responses to noxious stimuli in a model insect

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In addition to the well-characterized strike behavior in response to noxious stimuli, strong thermal stimuli to the body wall can trigger a rapid avoidance behavior (withdrawal) in Manduca larvae. Stimuli to the posterior abdomen elicit strikes and those to the head cause withdrawal, but both strike and withdrawal can be triggered by stimulating abdominal segments 2 and 3. Given that strike and withdrawal are mutually incompatible, there must be a decision-making process involved in nociceptive behavior. EMG recordings demonstrate that withdrawal movements are generated by rapid activation of contralateral ventral muscles. Ipsilateral ventral muscles are recruited at the end of the withdrawal behavior, presumably to control the extent of bending and to restore resting body posture. In contrast to their role in targeting strike behavior, the large intra-segmental dorsal muscles on each side of the midline are co-activated during withdrawal, which is consistent with lifting the anterior body segments away from the substrate. Both strike and withdrawal behaviors can be sensitized by prior stimulation and the likelihood of responding is reduced when Manduca adopts a "sphinx posture", suggesting that nociception is naturally modulated. Manduca therefore provides a unique opportunity to exam the neural mechanisms and ethology of nociceptive behavior.

Poster Session 1 | Poster Wall 134 | Label: PS1.134

Category: Motor systems, sensorimotor integration, and behavior

Investigating satiety-dependent decision making with quantitative behavioral analysis and molecular neuroanatomy in the a nudibranch specialist-predator

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Hunger and satiety can switch the valence of particular stimuli between attractive and repulsive. There are conserved signals for satiety and hunger in central nervous systems. The nudibranch Berghia stephanieae are specialist predators that feeds on a single species of anemone, Exaiptasia diaphana, which injures them every time they eat and can capture and kill them. Manual and machine learning approaches were used to quantify predatory behavioral changes in individual Berghia from 0 to 5 days of food-deprivation. Different phases of predation were differentially affected by hunger. All animals approached their prey regardless of food-deprivation length, indicating that the olfactory cues from their prey are always appetitive. However, the valence of contact cues from their prey appeared to be satiety-dependent. Thus, intermediately hungry animals had longer handling times and repeatedly updated their decision to approach or avoid their prey. More detailed analysis of responses to prey contact showed that the valence of responses on a contact-to-contact basis were somatotopic; the most important factor was where they were touched. This indicates that there is a complex interplay between satiety and the specific postures during predatory approaches. To investigate the potential signals of satiety and hunger in the brain, we used in situ hybridization-chain reaction to label expression of genes that are potential signals of hunger (NPF and feeding-circuit activating peptide) and satiety (the ortholog of allatostatin-A and insulins) in other animals. We identified the gene sequences for insulins in nudibranchs and identified the neurons that express these genes, determining whether there were state-dependent changes in expression. Taken together, we have begun to unravel mechanisms of state-dependent decision making in this nudibranch predator based on their behavior and molecular neuroanatomy.

Poster Session 1 | Poster Wall 135 | Label: PS1.135

Category: Motor systems, sensorimotor integration, and behavior

The role of two retinal regions in pecking of pigeons: a visual occlusion experiment

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Reach-to-grasp movement towards foraging targets is fundamental across species with different body anatomies. In primates, the separation of eyes (sensory organs) and bills (effectors) causes steady visual inputs. On the other hand, birds experience unsteady visual inputs during the reach movement, as their eyes and bills are in the head. Ground-feeding birds briefly stop their head towards the target, referred to as fixation, before starting their pecking. During fixation, birds could plan the pecking under stable visual inputs on the retina. Many birds have two retinal regions with differing depths of focus: the fovea centralis and the area dorsalis receive high-resolution visual inputs at closer and farther distances, respectively. Previous studies based on the observation of pecking behaviour in pigeons suggested that the visual image of the pecking target was projected onto the area dorsalis. However, the role of these retinal regions in pecking was not experimentally investigated. The present study examined the role of two retinal regions in pecking of pigeons by region-specific retinal occlusion with eyecaps. Given the hypothesis that the pecking of pigeons primarily relies on visual input of the target in the area dorsalis, we predicted that pigeons would fixate from a farther distance only when the area dorsalis was occluded by eyecaps. We set three different conditions in the available retinal region by the eyecap occlusion: fovea centralis-occluded, area dorsalis-occluded, and not-occluded control. Visual distance to the target, a bean seed, was measured to associate with the depth of focus of each retinal region. We found that pigeons usually fixate the target from a distance of 50–100 mm. As predicted, only when the area dorsalis was occluded, pigeons fixate from a relatively farther distance and with a longer duration. These results suggested that pigeons could use peripheral retinal regions instead of the area dorsalis at the fixation.

Poster Session 1 | Poster Wall 136 | Label: PS1.136

Category: Motor systems, sensorimotor integration, and behavior

Investigating motor-sensory signaling during Drosophila retinal movements

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Moving eyes pose a major challenge for the visual system: to an individual photoreceptor external world motion (exafferent signal) and self-generated motion input (reafferent signal) may look extremely similar. To disambiguate the visual input, visual neurons are often modulated by extraretinal signals, but our understanding of the cellular origins of these intricate signals remains limited.

Recent research has shown that fruit flies have the ability to move their retinas underneath the rigid lenses of their compound eyes. Notably, these retinal movements exhibit dynamics akin to vertebrate eye movements, such as fixational and nystagmus saccades. Leveraging the extensive genetic toolkit available for fruit flies, this discovery presents the unique opportunity to explore neuronal modulation during retinal movements.

Our study focuses on the modulation of horizontal (HS) and vertical system (VS) cells, which are optic flow-sensitive visual processing neurons.

Utilizing whole-cell patch-clamp recordings, we simultaneously record from HS/VS cells, monitor retinal movements and the behavioral state of the fruit fly. Preliminary findings indicate that HS and VS cells receive a motor-related input during retinal movements that is opposite in sign to the anticipated visual consequence, and therefore could serve as an efference copy.

To probe the potential origin of these motor-related potentials we explore the connectivity between the retinal motor system and the HS/VS cells, employing full brain connectome data as well as a neurobiotin labeling assay to explore both chemical and electrical connections. Through this integrated approach, we hope to enhance our understanding of cellular-level visual processing during eye movements.

Poster Session 1 | Poster Wall 137 | Label: PS1.137

Category: Motor systems, sensorimotor integration, and behavior

Deciphering Collective Motion: The Role of Visual Attributes in Locust Decision-Making

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Various mechanisms can serve visual-based decision-making, differing in their levels of complexity and computational demands. Accordingly, different visual attributes of objects in the environment can have relatively different roles and significance in such decision-making. A specific case is that of decisions regarding movement. In this respect, collective motion-related decisions present a particularly challenging case: the individuals need to synchronize their movements while within noisy and complex visual surroundings. We investigate the tentative role of optic flow—the motion pattern generated by the relative movements of the eye and the environment—in locust collective motion-related decision-making. An alternative second visual attribute tested, specifically relevant to collective motion, is that of the number of moving objects in the visual field. Using a well-established experimental setup, we present carefully controlled visual stimuli to individual locusts tethered in a natural walking posture, monitor and analyze their behavioral responses to stimuli differing in their specific visual characteristics: the number of discrete objects; the total pixel area; and the total pixels in the moving edge. Our findings suggest that the number of objects plays the most central role in the nymph's decision-making regarding collective marching. Reducing the number of objects to below a certain threshold affected the locusts' behavior even when the total number of pixels remained constant. It is the interplay between the number of objects and the moving-edge pixels that seems to determine the swarming-related behavioral response. These results offer novel insights into the fundamental visual-based mechanisms underlying information extraction and processing in a complex, cluttered social environment, specifically regarding the distinct attributes of the visual stimuli that are instrumental for collective motion-related decision-making in locusts.

Poster Session 1 | Poster Wall 138 | Label: PS1.138

Category: Motor systems, sensorimotor integration, and behavior

Variation and adaptiveness of locomotion in visually induced turning behaviour

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During locomotion, turning can be achieved through changes in various kinematic parameters such as stance direction, stride length or stride frequency. Even though the redundancy of a multi-legged motor system would allow a range of solutions, many current locomotion models have only a single mechanism that governs turning. Our aim was to investigate whether the leg movements that govern a turn of a given curvature are always the same, irrespective of what triggered the turn. To this end, we used a black bar (20° width) whose angular position on the arena wall was shifted instantly by either 60° or 120°. This shift was used to induce directed turning behaviour in freely walking stick insects (Carausius morosus). Visual stimuli were projected on a screen surrounding a circular open field with a diameter of 120 cm. Walking behaviour was captured using overhead motion capture and marker-less tracking.

Generally, we found that, without visual guidance, animals tended to walk around in circles and that the size of the position shift affected the likelihood of turning. Interestingly, the initial turning responses, i.e. rotational acceleration, to a shift in landmark position scaled non-linearly with the size of change in landmark position, whereas the magnitude of the turning response did not.

Overall, we show that kinematic parameters of leg movements during visually guided turns are less variable compared to spontaneous turns. Furthermore, behavioural responses, as displayed in rotational velocities and general trajectories were linked to the magnitude of change in visual information.

Poster Session 1 | Poster Wall 139 | Label: PS1.139

Category: Motor systems, sensorimotor integration, and behavior

Overlapping muscles drive multimodal courtship signals in Drosophila

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The brain coordinates body movement by processing sensory cues, selecting from various motor programs. Muscle activity is therefore the ultimate output of neural computation. However, with fewer muscles than behaviors, it remains unclear how overlapping sets of muscles produce distinct motor outputs. Here, we use courtship signaling in Drosophila to study this issue. During courtship, males select between two signal types: air-borne song and substrate-borne vibrations. The song occurs in bouts and consists of two modes: Sine song corresponds to sustained tones. Pulse song consists of trains of short pulses produced at regular intervals (40ms). Substrate-borne vibrations are also pulsatile but have longer intervals (150ms). Song results from unilateral wing fluttering, controlled by well-understood mechanisms: indirect wing muscles create power and direct wing muscles fine tune the song. Vibrations are not produced by wings and are correlated with abdominal movement. However, males without abdomen still vibrate. Therefore, how vibrations are produced remains unclear. We hypothesize that both song and vibration pulses originate from the thorax, with vibrations transmitted through the legs to the substrate. Due to the limited thoracic muscles, overlapping muscles likely produce both signals. To test this, we deactivated wing muscles controlling song and examined changes in signal structures. We find an extensive overlap between the muscles that drive song and vibration. Inactivating the indirect DLMs abolishes vibrations while it softens song. In addition, the direct wing muscle i2 shaped frequency and intervals of song and vibration pulses whereas the b2 muscle impacts vibration amplitude. We are combining connectomics with genetic tools to identify networks that control wing muscle patterns. In addition, we will perform calcium imaging to assess the dynamical engagement of muscles throughout the body – for instance in legs and abdomen – during song and vibration.

Poster Session 1 | Poster Wall 140 | Label: PS1.140

Category: Motor systems, sensorimotor integration, and behavior

Analyzing individual locomotion behavior in Drosophila larvae

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Behavioural variability in genetically identical backgrounds is known for Drosophila adults where object responses in a Buridans paradigm correlate with asymmetric or symmetrical wiring in Dorsal Cluster Neurons (Linneweber et al., 2020). In addition, handedness in flies was shown to differ on the population level in Drosophila Genetic Reference Panel (DGRP) flies, where the activity of a subset of PFN neurons in the central complex modulates the more extreme handedness phenotypes (Buchanan et al., 2015). Curiously, these differences are not heritable, therefore not solely based on genetics and could not be explained by morphological differences.

Interestingly, also in Drosophila larvae there seems to be phenotypic variability in behavioral responses. Larval forward and backwards locomotion consists of peristaltic muscle contraction either from posterior to anterior (forwards movement) or anterior to posterior (backwards movement) (Heckscher et al., 2012). During reorientation, the larva stops while the head sweeps from left to right to sample sensory information in a temporal as well as spatial manner (Humberg et al., 2018). Looking not only at the locomotion but at the overall movement pattern, hence the trajectories of individual larvae, there seems to be variability in their overall locomotion. Trajectories of crawling Drosophila larvae are characterized by active movement and reorientation phases. But even in the absence of external stimulation, two strategies of reorientation can be observed. Firstly, a straight crawl - stop - head sweep - reorientation followed by a straight crawl. Secondly, a clockwise or counter-clockwise circling of the area. These strategies are observed for different individuals of the same isogenic background as well as well as for wild type strains like Canton S and Berlin K. Here we tested for individuality in larval locomotion behavior and aim to test if behavioral patterns persist after the metamorphosis.

Poster Session 1 | Poster Wall 141 | Label: PS1.141

Category: Motor systems, sensorimotor integration, and behavior

Load feedback in fruit fly walking: analysis of sensory projections and role in generating leg stepping and motor flexibility

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Fruit flies, Drosophila melanogaster, are vigorous walkers with a wide range of walking speeds. Similar to other walking insects, feedback signals from sense organs on the legs, such as campaniform sensilla (CS), are likely to contribute to the motor control of walking. CS detect changes in load via cuticular strains. The CS signals are conveyed into the ventral nerve cord (VNC), the insect's equivalent of the spinal cord, but it is not known how they affect walking behaviour, e.g. walking speed. Previously, it was shown that axons of somatosensory cells arborise in the VNC in a modality-dependent fashion (Tsubouchi et al. 2017). However, even with recently released connectome datasets, data on the arborisation patterns of CS are scarce. Our study addresses these issues: (i) we determine axonal projections of leg CS neurons, using a stochastic labelling method (MultiColor FlpOut – MCFO; Nern et al. 2015) in the VNCs and legs of the corresponding flies. This allowed us to unravel the projection patterns of single CS. As a result, we report that it is possible to generalise location-specific arborisation patterns of CS neurons. However, we also observe special cases of CS neurons with unique projection patterns. This might indicate a distinct influence on the motor output. (ii) To address this issue, we investigated the influence of CS on walking, using optogenetic inhibition of CS neurons in freely-walking flies. We show that inhibiting subsets of CS neurons change leg stepping kinematics, walking speed distribution, the duration of walking bouts, and walking frequency. We are currently investigating whether the observed effects are related to individual CS within a group and correlate with their arborisation pattern and connectivity. Identifying local circuitries in the VNC will help in understanding how the locomotor output is being adapted to the requirements of the behavioural goals.

Poster Session 1 | Poster Wall 142 | Label: PS1.142

Category: Motor systems, sensorimotor integration, and behavior

Kinematic synergies of leg stepping in straight-walking fruit flies, Drosophila melanogaster

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Because biological limbs have a large number of mechanical degrees of freedom (DOFs), the nervous system must cope with the problem of coordinating more available joint DOFs than would be required to perform stepping during walking. However, the observation that joint movements are commonly coupled during the execution of motor tasks led to the hypothesis that single DOFs are controlled by the nervous system in groups called synergies. Here, we aimed to identify kinematic synergies for stepping in straight-walking fruit flies, Drosophila melanogaster, by exploiting the covariation between the angle time courses of the leg joints. For this, fruit flies (n= 12) walked on a spherical treadmill and were recorded with six synchronized high-speed cameras (400 Hz). Positions of leg and body keypoints were tracked by using DeepLabCut and triangulated to obtain 3D reconstructions. Additionally, we developed a kinematic leg model based on micro-computed tomography (µCT) scans to extract accurate joint angles and to reconstruct leg postures from the identified kinematic synergies.We found that stepping kinematics of the front, middle, and hind leg pairs differed in terms of joint DOFs employed and angle time courses. To identify kinematic synergies, we performed principal component analyses (PCAs) on the average angle time courses for each leg pair of each fly. The first three PCs cumulatively captured over 97% of variance for all leg pairs in all flies and their coefficients and the scores' time courses were broadly similar across animals. Strikingly, these PCs were sufficient to reconstruct the movements of the tarsus tip with high accuracy. In conclusion, our results show that only three kinematic synergies can sufficiently capture the coordination between leg joints in walking fruit flies. Moreover, the consistency of the kinematic synergies found here between flies and leg pairs suggests that stepping may be controlled by generic motor activation patterns in Drosophila.

Poster Session 1 | Poster Wall 143 | Label: PS1.143

Category: Motor systems, sensorimotor integration, and behavior

Multisensory integration of aversive visual and auditory cues in Aedes aegypti mosquitoes

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Mosquitos pose significant challenges for public health, motivating study of their life history and sensory biology. Blood-feeding Aedes aegypti mosquitoes face swatting risks from potential hosts, and both male and female mosquitoes face predation from aerial predators such as bats, dragonflies, and robber flies. While prior research has characterized visually-evoked escape responses to these threats, (Wynne et al. Scientific Reports, 2022), how do mosquitoes respond to auditory cues that may signal the presence of predators and how are auditory and visual cues integrated? Lapshin and Vorontsov (Entomological Review, 2018) identified a negative phonotaxis to 140-200Hz sounds in free-flying Aedes diantaeus mosquitoes. This frequency range encompasses the wingbeat frequencies and low-order harmonics of aerial predators such as dragonflies and robberflies, suggesting that mosquitos may use their sensitive hearing (well-characterized for its role in conspecific signaling) to escape from predators as well. Here, we expand upon these findings, using a tethered flight paradigm to quantify physiological responses and behavioral changes in head yaw, head roll, wing thrust and wing frequency following presentation of a range of low-frequency stimuli in Ae. aegypti. We compare these responses with those evoked by looming visual stimuli, and with those evoked by concurrent low frequency sounds and visual looms, outlining multisensory control over responses to threatening stimuli.

Poster Session 1 | Poster Wall 144 | Label: PS1.144

Category: Motor systems, sensorimotor integration, and behavior

Parallel sensorimotor pathways control landing in Drosophila

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Landing is the final and arguably most critical stage of flight. To avoid impact injuries, animals decelerate by modulating wing and body kinematics and extend their legs in a well-coordinated movement sequence before touchdown. This is a challenging motor task, which depends on the integration of different sensory modalities, in particular visual cues. However, how visual cues are integrated by peripheral and central networks in the brain and conveyed to lower-level motor networks in the ventral nerve cord (VNC) to control landing is largely unknown.

Here, we analyzed the distributed control of landing in the nervous system of Drosophila. Previous work has characterized early visual processing circuits, the landing behavior, and two descending neurons (DNs) controlling landing. Using a combination of light microscopy and connectomics approaches, we now identified complete neuronal pathways for landing from the brain to motor neurons in the VNC. By combining genetic activation and silencing with behavioral tracking, we then validated their functionality. We identified four classes of visual projection neurons (VPNs) that consistently drove landing upon optogenetic activation. Silencing three of these significantly impaired visually evoked landing responses. Hence, these VPNs are core components of the landing circuitry. The VPNs synapse directly onto a population of DNs which project to motor circuits in the VNC. Activating different VPNs and DNs drove landing responses with distinct leg, body, and wing kinematics. Finally, we used a connectome of the VNC to identify novel DNs based on their shared synaptic outputs in the VNC with previously described landing DNs. Different types of landing DNs recruit distinct sets of leg and wing motor neurons and drive different landing responses. Together, our findings elucidate parallel sensorimotor pathways from the brain to the VNC that enable flexible landing responses involving coordinated movements of all body appendages.

Poster Session 1 | Poster Wall 145 | Label: PS1.145

Category: Motor systems, sensorimotor integration, and behavior

Analyzing the odor source localization behavior of adult male silkmoth, Bombyx mori, in response to hierarchical environmental complexity

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Insects acquire a diverse array of information via numerous sensory organs and execute requisite tasks by selecting and modulating pertinent actions. To cope with various situations, insects possess a robust sensory-motor integration mechanism. In this study, we analyze the multisensory-motor integration system of the adult male silkmoth, bombyx mori, when the complexity of the environment is varied stepwise. As environmental complexity fluctuates, it is anticipated that both the quality and quantity of information obtained from multisensory organs will undergo significant changes, necessitating corresponding behavioral modulations in the silkmoth.

To investigate this, we measured the localization behavior of the silkmoth amidst hierarchical variations in environmental complexity, employing a teleoperation experimental framework. This framework consists of a behavior measurement device that records behavior while providing multiple sensory stimuli, and an agent that moves around the real environment in place of the silkmoth. Sensory stimuli presentation conditions are determined according to the values of the sensors mounted on the agent; therefore, it perceives the environment through the agent's body. Utilizing this framework, experiments were conducted across three tiers of environmental complexity: virtual, indoor, and outdoor settings.

The outcomes of the odor source localization experiment unveiled a search success rate exceeding 95% within virtual and indoor environments, whereas it diminished to 70% within the outdoor environment. To provide a quantitative assessment of the search trajectory, we computed straightness as a metric. As a result, within the outdoor environment, the silkmoth exhibited a propensity to veer more toward the crosswind direction similar to flight insects. This suggests that it may switch to a strategy of moving in a crosswind direction to increase the probability of encountering odors as the environment becomes more complex.

Poster Session 1 | Poster Wall 146 | Label: PS1.146

Category: Motor systems, sensorimotor integration, and behavior

Marmoset monkeys use different avoidance strategies to cope with ambient noise during vocal behavior

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Multiple strategies have evolved to compensate for masking noise, leading to changes in call features. One call adjustment is the Lombard effect, an increase in call amplitude in response to noise. Another strategy involves call production in periods where noise is absent. While mechanisms underlying vocal adjustments have been well studied, mechanisms underlying noise avoidance strategies remain largely unclear. We systematically perturbed ongoing phee calls of marmosets to investigate noise avoidance strategies. Marmosets canceled their calls after noise onset and produced longer calls after noise-phases ended. Additionally, the number of uttered syllables decreased during noise perturbation. This behavior persisted beyond the noise-phase. Using machine learning techniques, we found that a fraction of single phees were initially planned as double phees and became interrupted after the first syllable. Our findings indicate that marmosets use different noise avoidance strategies and suggest vocal flexibility at different complexity levels in the marmoset brain.

Poster Session 1 | Poster Wall 147 | Label: PS1.147

Category: Motor systems, sensorimotor integration, and behavior

Vocalization-correlated neural responses in the marmoset brainstem

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The execution of vocalizations relies on the coordination of cortical and brainstem neural networks. In monkeys, cortical regions are primarily involved in the initiation of call production, while the complex acoustic features of vocalizations are generated at the brainstem level. The vocal pattern generator in the brainstem generates the neural patterns necessary to drive all the motoneuron pools involved in call production. Using a semi-chronic electrophysiological recording approach, we investigated the role of the vocal motor network in the brainstem of marmoset monkeys producing volitional vocalizations within a visual detection task. We found single neurons with vocalization-related activity in all the recorded motoneuron pools involved in vocal output as well as in the putative vocal pattern generator. The neural activity patterns reveal a complex brainstem network involved in vocal motor control.

Poster Session 1 | Poster Wall 148 | Label: PS1.148

Category: Motor systems, sensorimotor integration, and behavior

What came first, the flicker or the firing rate? Links between salient stimulus features and neural encoding in predatory Asilidae Flies

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Many aerial predatory invertebrates rely on specific visual cues to initiate and maintain pursuit, such as target speed and size (Wardill et al., 2015). However, recent work by Talley et al. has shown that other stimulus features such as stimulus flicker rate are correlated with predatory pursuit behavior in robber (Asilidae) flies. As robber flies exhibit a range of prey preferences, it is hypothesized that distinct visual features are behaviorally salient for different genera of Asilidae. Furthermore, premotor encoding of visual targets is expected to vary with feature salience. In order to explore these questions, we recorded the activity of premotor descending neurons (TSDNs) in five genera of Asilidae flies (Laphria, Neoitamus, Efferia, Diogmites, Protocanthus) while presenting prey-like visual stimuli with different contrasts and flicker rates.

TSDN activity varied among Asilidae genera when presented with a white target on a black background vs a black target on a white background. Laphria TSDNs exhibited more spiking activity with a white target while Diogmites, Efferia, and Neoitamus TSDNs produced more action potentials in response to a black target. Target flicker rate also had an effect on TSDN activity in some genera. In Laphria, Neoitamus, and Diogmites, the 120Hz stimulus was correlated with more TSDN spikes while the 360Hz stimulus was more salient for Efferia TSDNs. Certain TSDNs in Laphria and Neoitamus exhibited periodic activity matching the flicker rate of the stimulus. Temporal analysis of these trials suggests that target position encoding is linked to the flicker rate of the stimulus.

Talley, J., et al. (2023). Predictive saccades and decision making in the beetle-predating saffron robber fly. Current Biology, 33(14).

Wardill, T. J., et al. (2015). The Killer Fly Hunger Games: Target size and speed predict decision to pursuit. Brain, Behavior and Evolution, 86(1), 28–37.

Poster Session 1 | Poster Wall 149 | Label: PS1.149

Category: Motor systems, sensorimotor integration, and behavior

Functionality of the regenerated nervous system

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Regeneration is not only about shape and form but also about function. Recently, there has been enormous progress in defining the neuronal circuits that control movement thanks to newly established methods like molecular genetic analysis, GCaMP imaging and optogenetics. This provides the opportunity to bring together analysis of tissue patterning with functional circuit neuroscience, and I aim to address: How accurate is organ regeneration in terms of function?

The axolotl has unique regenerative capabilities, and the axolotl tail is particularly well suited to study regeneration of the spinal cord and locomotor-related spinal circuits, as it is non-essential, yet possesses all relevant structures found higher up the body axis. In particular, I aim to investigate the patterning and functionality of the regenerated nervous system on multiple levels from sensory input to activity to motion: (1) projection patterns, connectivity, and muscle innervation; (2) swimming behaviors and activity of neurons within and across muscle segments; and (3) sensory input into central pattern generators (CPGs). Volumetric imaging showed that the muscle tissue is not well segmented after regeneration. However, even though segmentation is not perfect, a transgenic CAGGS-GCaMP reporter revealed that regenerated muscle fibers are highly active when induced with neurotransmitters like Acetylcholine or N-methyl-D-aspartate. In addition, swimming ability was restored already 3 weeks post amputation, and when the tails reached the length of their age-matched controls (6 wpa), no obvious differences in S-wave motions were detected. Preliminary results showed that even if patterning is not fully reestablished, the regenerated neuronal network is functional. In the future, this study will shed light on how accurate organ regeneration needs to be to still be functional, and point the way for a more detailed investigation of patterning and connectivity in less regenerative mammalian species.

Poster Session 1 | Poster Wall 150 | Label: PS1.150

Category: Motor systems, sensorimotor integration, and behavior

Temperature resilience of the pyloric and gastric neuromuscular systems

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Temperature influences all biological processes and presents a challenge in maintaining stereotyped activities within neuronal circuits. Our study focuses on the crab Cancer borealis, which experiences daily and seasonal temperature (T°) fluctuations. Its stomatogastric system, comprising the pylorus and the gastric mill—responsible for filtering and grinding food—has been a key testbed for over 50 years, characterized by well-identified neuronal circuits and muscles. Recent research has revealed that as T° increases, the pyloric rhythm frequency rises while retaining its characteristic phase until it "crashes" at high T°. Neuromodulators such as oxotremorine (OXO) and proctolin (Proc) enhance rhythm robustness, whereas serotonin (5-HT) diminishes it. In this project, we build upon prior work by exploring the impact of T° on the neuromuscular junction and comparing different muscles (p1, p2, p8, gm5, gm6) of the same species. Initially, we record muscle fibers driven by spontaneous pyloric rhythm activity, gradually increasing the T° until a crash occurs. This approach illuminates the "weak-link" between nerve and muscle under T° stress. Additionally, nerve-evoked excitatory junction potentials (EJP) and excitatory junction currents (EJC) are obtained by stimulating the nerve (1 to 5Hz for 1s every 2s) and a Poisson train (with an average frequency varying from 1-10Hz). We quantify synaptic properties with increasing T°, observing a decrease in membrane resistance and resting membrane voltage, and a decrease in EJP changes in facilitation and depression. Through comparisons of various pyloric and gastric muscles, we aim to develop a comprehensive understanding of the system. Finally, we examine how neuromodulators such as dopamine, 5-HT, OXO, and Proc influence muscle T° sensitivity. Understanding the nervous system response to climate change is crucial and plays a pivotal role in raising awareness about the impact of the current crisis on neurological functions.

Poster Session 1 | Poster Wall 151 | Label: PS1.151

Category: Motor systems, sensorimotor integration, and behavior

Optogenetic antennal stimulation drives haltere movement in Drosophila

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Insect flight, with wing flapping frequencies up to 1000Hz, requires rapid sensory processing of multiple stimuli. In flies, information for flight control comes primarily from the eyes, the antennae, and the mechanosensory halteres, but how these systems function in concert is unresolved. Recent work showed that visual input to the haltere's steering muscles changes their activity and thus the haltere's motion. This change in motion, in turn, changes the sensory input from the halteres to the wing and neck motoneurons. Are other sensory modalities integrated to drive haltere movements and thus modulate haltere sensing?

Here, we used optogenetics to stimulate antennal muscles in tethered flies. Simultaneously, we used high-speed cameras to closely observe haltere motions. We found that some, but not all, movements of the antenna cause changes in the amplitude of the haltere during flight, and drive haltere movement in quiescent flies. These results suggest a specific and modifiable link between the antennae and haltere that allows them to act as interconnected sensors. They also provide further evidence that halteres function not only as passive sensors for detecting body rotations, but are also under active control from multiple other senses.

Poster Session 1 | Poster Wall 152 | Label: PS1.152

Category: Motor systems, sensorimotor integration, and behavior

Exploring the role of a premotor cell type for active sensor control in Drosophila

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An animal's nervous system enables it to detect and respond to stimuli to navigate its environment. To enhance sensory acquisition, animals can actively position sensors, altering how they extract information from the external world. However, active sensing, and movement in general, produces stimuli that feeds back onto these same sensory systems – requiring mechanisms for integrating predictive motor signals with externally-generated sensations. Despite the importance of these mechanisms for guiding coordinated behavior, the cellular and circuit basis of motor control and sensation during active movements are not fully understood. Here, I use optogenetics, electrophysiology, and machine-learning assisted antennal tracking to investigate the role of a class of genetically-identified second-order antennal mechanosensory neurons in positioning the antennae in Drosophila. Preliminary connectomic data reveals a synaptic connection between this neuronal cell class and antennal motor neurons, while optogenetic activation experiments suggest a role for this cell class in controlling antennal movements. To explore this further, I am using whole-cell patch clamp electrophysiology to measure the relative influence of sensory input on this cell class during behavior. Together, this work aims to uncover the functional logic of a sensory-motor circuit controlling an active sensor.

Poster Session 1 | Poster Wall 153 | Label: PS1.153

Category: Motor systems, sensorimotor integration, and behavior

Experience dependent modulation of collective behavior in larval zebrafish

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Complex collective behaviors such as schooling in fish and flocking in birds are crucial for animal survival, providing protection from predators and enhancing foraging and navigation abilities. These large-scale group patterns emerge from simple, local inter-individual interactions. Commonly, these interactions are considered static and hardwired, and little is known about how experience and learning affect collective behaviors. Previously, we mapped the sensory-motor transformations that govern collective swimming behavior in larval zebrafish throughout their development and described the neural circuits involved in these computations (Harpaz et al., 2021). Here, we explored how groups of animals dynamically modify their collective behaviors to adapt to changing social conditions. We found that larval zebrafish modulate their inter-individual interactions and resulting collective behaviors in response to acute changes in their population density. Using naturalistic and virtual reality experiments, we show that fish swimming in a specific group density will exhibit weaker (stronger) interactions if they were previously exposed to higher (lower) neighbor densities. These adaptations gradually develop over tens of minutes. Mechanistically, we show that larvae estimate their group density by temporally integrating changes in visual occupancy and couple the strength of their interactions to that estimate. A dynamic state-space model, which adjusts agents' social interactions based on past experiences, accurately describes our behavioral observations and predicts novel aspects of behavior that were confirmed in subsequent experiments. These findings provide concrete evidence that inter-individual interactions are not static, but rather continuously evolve based on experience and current environmental demands. The underlying neurobiological mechanisms of experience-dependent modulation of collective behavior can now be explored in this species.

Poster Session 1 | Poster Wall 154 | Label: PS1.154

Category: Motor systems, sensorimotor integration, and behavior

Comparative physiology of the mosquito and fruit fly wing motors

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Flying insects are capable of remarkable feats of aerial agility. For insects like Diptera (true flies), this prowess is largely enabled by a suite of biomechanical and physiological innovations at the wing hinge. There, a series of tiny steering muscles insert onto an intricate system of hardened skeletal elements at the base of the wing and modulate the wingstroke to actuate both rapid maneuvers and stabilization control. The functional organization and neural control of this wing motor system have been studied extensively in the fruit fly, Drosophila melanogaster, and the blowfly, Calliphora vicina, both of which are members of the relatively recent cyclorrhaphan radiation. Much less is known, however, about the control of wing movement in more basal groups such as mosquitoes, whose low-amplitude, high-frequency wing kinematics are qualitatively different from those of both blowflies and fruit flies. How do mosquitoes implement neuromuscular control of this distinct mode of flight using the largely conserved Dipteran wing motor system? Here, we present first steps towards a characterization of the flight motor control system in the mosquito, Aedes aegypti. We record extracellularly from wing muscles in flying mosquitoes, correlate muscle activity to wing kinematics obtained from high-speed videography, and compare these findings to the measured activity of homologous motor elements in Drosophila. We also explore the use of transgenic mosquitoes expressing the genetically encoded calcium indicator, GCaMP, in wing muscles to simultaneously monitor the activity of the full flight motor. This work serves as a foundation for future comparative efforts aimed at understanding the evolution of the Dipteran flight system, as well as insect flight more broadly.

Poster Session 1 | Poster Wall 155 | Label: PS1.155

Category: Motor systems, sensorimotor integration, and behavior

Developmental differentiation of song-related neural activity in the basal ganglia of Bengalese finches

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Humans are unique among other mammals in the ability to learn complex motor sequences such as speech. Similarly, songbirds spontaneously learn motor sequences known as songs without external rewards. Examining the neural basis of song learning would help understanding the emergence of speech in humans. The basal ganglia are crucial brain structure for song learning. These areas contain neurons with singing-related activities (Goldberg & Fee, 2010). Songbirds which received lesions in the basal ganglia could not learn the structure of song sequences properly (Sohrabji et al., 1990). Based on these findings, we hypothesized that developmental changes in neural activity in the basal ganglia should contribute to the processing of song motor sequences. To examine this, we used adult and juvenile Bengalese finches and recorded the neural activity of the basal ganglia while the birds were singing. We then compared the singing-related activities of the neurons between adults with fully crystallized songs, and juveniles with plastic songs. In adult finches, we found neurons with singing-related activities exhibiting tonic or phasic firing patterns. Specifically, some neurons exhibited a phasic response in firing rate at the onset or offset of songs, while other neurons showed responses at the specific timing of all syllables. In contrast, most neurons of juvenile birds showed tonic responses rather than phasic responses. These results suggest that, during motor sequence learning, the basal ganglia neurons initially process the motor sequences as a whole, while some neurons gradually develop syllable selectivity. We suggest that the basal ganglia neurons play an important role in refinements of motor sequences during the learning process. (Work supported by JSPS grant JP23H05428 & JSPS DC Research Fellowships.)

Poster Session 1 | Poster Wall 156 | Label: PS1.156

Category: Motor systems, sensorimotor integration, and behavior

Cortical representation of facial features and body posture in freely moving rats

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Understanding how the brain produces and orchestrates behavior is one of the core aims of neuroscience. Our group studies these topics in rats, utilizing recent advances in tracking technology and analytic tools to quantify and explore behavior in naturalistic, unrestrained contexts. Previous publications from the group describe robust postural and behavioral representations in the posterior parietal cortex (PPC) and frontal motor cortex (Mimica et al., 2018) as well as primary sensory cortices (Mimica, Tombaz et al., 2023). To better understand how neuronal populations integrate active sensory input to inform and update ongoing posture, we are now expanding our recording paradigm to include tracking of whiskers and other facial features. We therefore developed a custom head-mounted set-up combining two facial-tracking cameras, housing for Neuropixels recording probes (allowing for insertion at relevant angles), and a retroreflective rigid body for 3D posture tracking. We are currently employing this approach to investigate sensory-motor integration in two cortical areas. Firstly the barrel cortex, i.e. the region of primary somatosensory cortex (S1) associated with whiskers, where our preliminary analyses confirm stable encoding of whisker position. Our second area of interest remains the PPC where we hope to extend existing work on behavioral representation in the region by investigating the combined representation of multiple body schema effectors (e.g. the head, vibrissae, and eyes) during spontaneous behavior.

Poster Session 1 | Poster Wall 157 | Label: PS1.157

Category: Motor systems, sensorimotor integration and behavior

Sleep disruption improves behavioral performance in zebrafish larvae

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Sleep deprivation is known to drastically affect cognitive function including decision-making and attention across many different species. Here, we leveraged the small size and conserved brain structure of larval zebrafish to investigate the consequences of sleep disruption in the context of two well-described behaviors, a visual and an olfactory-based decision-making task. We find that in both paradigms, sleep disruption leads to an improvement in performance. Specifically, we show that sleep disruption increases reaction time and improves performance in a visual motion discrimination task, an effect that we attribute to longer integration periods in disturbed animals. We also find that sleep disruption leads to increased odor sensitivity, which we show is likely mediated by cortisol. Building on our previous research, our work allows us to predict specific circuit changes underlying these effects. Our findings set the groundwork for further investigation of the underlying circuit changes in the brain that occur as a result of sleep disturbance across different species.

Poster Session 1 | Poster Wall 158 | Label: PS1.158

Category: Motor systems, sensorimotor integration, and behavior

Shared Neural Substrates for Seasonal and Evolutionary Shifts in Sensorimotor Integration

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Mormyrid fishes communicate using electric organ discharges (EODs). Electroreceptors specialized for communication respond to both self-generated (reafferent) and externally generated (exafferent) EODs. To distinguish between reafferent and exafferent EODs, an internal copy of the EOD command, termed corollary discharge (CD), signals the timing of EOD production. CD briefly inhibits central electrosensory neurons to block responses to reafferent EODs. EOD duration is diverse across species, and CD timing has evolved to maintain a precise match between the timing of inhibition and reafferent sensory input. Similarly, seasonal increases in testosterone reversibly elongate male EODs, and testosterone shifts CD timing in the brain to match the resulting shift in reafferent feedback. The mechanisms that keep CD precisely time-locked to reafferent input remain unknown. Here, we identify sites of both seasonal and evolutionary shifts in the timing of CD activity within this circuit. We treated Brienomyrus brachyistius with testosterone to elongate EODs and induce CD plasticity. We sequentially recorded field potentials from six nuclei linking electromotor, CD, and electrosensory pathways. We discovered that testosterone delayed synaptic input and elongated field potentials in the mesencephalic command-associated nucleus (MCA) of the CD pathway, which shifted downstream activity relative to controls. We recorded from these same areas in two Campylomormyrus species: one with short-duration EODs (~0.2 ms), and one with long EODs (2-25 ms). We found long-EOD fish also had elongated MCA activity relative to short-EOD fish. Additionally, long-EOD fish showed delayed MCA activity relative to short-EOD fish, but unlike testosterone-treated fish, delays appeared to originate in MCA neurons rather than from delayed presynaptic input. These results reveal common substrates underlying CD shifts but suggest distinct mechanisms operating on seasonal and evolutionary timescales.

Poster Session 1 | Poster Wall 159 | Label: PS1.159

Category: Motor systems, sensorimotor integration, and behavior

Sticking the landing: unraveling mechanisms of proprioceptive feedback in flying insects

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Performing a successful landing is arguably the most important step of flight. When landing, flying animals must quickly and precisely coordinate multiple limbs to transition to a stable standing posture, often on surfaces varying in stability, texture, and orientation. Proprioceptive sensory neurons on the legs signal surface contact and the distribution of body load across the legs. These neurons are therefore likely key to coordinating successful landings, but have yet to be studied.

We have developed a novel behavioral assay using the fruit fly, Drosophila melanogaster, to determine how proprioceptive feedback coordinates limb movements during landing. We induce landing in tethered, flying flies by elevating a platform to contact their legs, mimicking the contact that occurs during landing. We then track the 3D kinematics of leg joints, wings, and body of flies as they respond to this contact. By adjusting its speed, size, or location, we are dissecting how mechanical perturbation of the legs influences landing. Flies are more likely to land and do so with a lower latency when the distal tibia-tarsus leg joint is contacted compared to the proximal coxa-trochanter-femur leg joint. Additionally, the coordination pattern of each leg changes depending on which leg is contacted.

We are also determining which sensory neurons underlie this landing response. The fly ventral nerve cord (VNC), the invertebrate analog of the vertebrate spinal cord, mediates leg and wing sensation and motor control. Connectivity analyses from a synapse-resolution volume of the fly VNC suggest that strain-sensing campaniform sensilla (CS) mediate the fly's detection of the platform and subsequent cessation of flight. We are currently developing tools to target leg CS to determine how their activity affects the transition from flight to standing. Altogether, these experiments will provide novel insights into how proprioceptive feedback rapidly coordinates ethologically important behaviors.

Poster Session 1 | Poster Wall 160 | Label: PS1.160

Category: Motor systems, sensorimotor integration, and behavior

Intrinsic connectivity of a multipurpose central pacemaker nucleus in Gymnotus omarorum

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In Gymnotiformes, the medullary pacemaker nucleus (PN) commands the electric organ discharge (EOD), whose rhythmic discharge can be modulated to cope diverse environmental and behavioral demands. The PN is composed of segregated subpopulations of pacemaker cells (PMc) and relay cells (Rc) connected in a feedforward manner. Rc send the descending command to lower levels of the electromotor system to emit stereotyped EODs. Modulatory inputs to PN neurons cause electromotor behaviors with different adaptive significance depending on its specific cellular target (PMc or Rc). Electrotonic coupling (EC) between PN neurons has been proposed as a critical functional characteristic of the intrinsic connectivity of the PN. To test whether EC is capable to guarantee the many functional requirements of the PN as a robust pacemaker and a flexible effector of a wide electromotor repertoire, intrinsic connectivity of the PN of Gymnotus omarorum was studied in brainstem slices of juveniles using electrophysiology, pharmacology and immunohistochemistry and evaluation of dye coupling between PN neurons. PMc-PMc and Rc-Rc pairs showed low magnitude EC through non-rectifying contacts with a low-pass filter behavior. At the PMc, this could contribute to a robust yet modifiable pacemaker behavior of the PN; at the Rc would be suitable for the generation of a synchronous descending command. Weak PMc-Rc EC was also detected. This connection was non-rectifying and bidirectional but with direction-dependent filter behavior: high-pass in the PMc-Rc direction and low-pass in the Rc-PMc direction. Directionality and adequate timing of PMc and Rc activation would result from the existence of EC through axo-dendritic or axo-somatic connections and special anatomical characteristics of the PMc axon. The effect of specific blockers, the presence of dye coupling between PN neurons and immunohistochemical results suggest that neurons of the PN are connected via gap junctions probably formed by Cx35.

Poster Session 1 | Poster Wall 161 | Label: PS1.161

Category: Motor systems, sensorimotor integration, and behavior

Understanding the impact of early microexon misregulation on zebrafish sleep/wake behaviour

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The need to match sensory input with appropriate behavioral responses is highly widespread across the animal kingdom. Altered neurodevelopment can disrupt such tightly regulated and frequently state-dependent responses. This study looks into a highly conserved and neuron-specific splicing program of microexons (3-27bp) important during late neurogenesis responsible to fine-tune crucial processes such as synaptogenesis and axon guidance, actively contributing to an optimal excitation/inhibition balance in the brain (Irimia et al. 2014; Quesnel-Vallières, et al. 2015, Nakano et al. 2019). However, little is known about how misregulation of microexons during development can result in a shift in brain state and key signaling cascades as well as locomotor output. We have set out to investigate this for the mutant of the microexon splicing master regulator srrm3, a gene misregulated in autism spectrum disorders (ASD). We first tacked zebrafish's sleep and wake behavioral as well as their relatively complex but stereotyped repertoire of movements (bouts) (Marques et al. 2018). Our observations suggest for a multi-facetted hyperactivity phenotype with sleep loss at night, characterized by an altered in bout type usage as well as ASD-like symptoms such as increased anxiety, repetitive behaviors and increased sensitivity to external stimuli. These behavioral symptoms were accompanied by an excitation/inhibition imbalance in the zebrafish larvae brain as seen via calcium imaging and pharmacological experiments. More specifically, we use behavioural pharmacology to further disentangle signalling pathways affected by early microexon misregulation and found that altered levels of the secondary messenger cAMP can in explain some of the behavioural hyperactivity observed in the mutant. With this research we shed new light into the functional impact of microexons on brain development and contribute to our understanding on neurodevelopmental neuoplasticity in zebrafish larvae.

Poster Session 1 | Poster Wall 162 | Label: PS1.162

Category: Motor systems, sensorimotor integration, and behavior

Male and female syringeal muscles exhibit superfast shortening velocities in zebra finches

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Vocal production requires precise motor control, which is executed by superfast vocal muscles. These muscles can operate at cycle frequencies over 100 Hz and up to 250 Hz. The mechanical performance of muscle is characterised by isometric (Force-length; F-L), force-velocity (F-V) and cyclic performance. In syringeal muscles we lack a key muscle property characterising muscle performance: the F-V relationship. Here we measure the F-V relationship of a zebra finch syringeal muscle using the isovelocity technique. We measured the force output of syringeal muscle at velocities from 17.5 to 0.25 L0 s-1 at 20 and 30°C and estimated function at 40°C using a Q10-based extrapolation. Syringeal muscles exhibited high shortening velocities of 25L0 s-1 at 30°C and are estimated to reach between 37-42L0 s-1 on average at body temperature, exceeding other vocal and non-avian skeletal muscles. Furthermore, we show that isometric properties positively correlate with maximal shortening velocities. Although male and female muscles differ in isometric force development rates, maximal shortening velocity is surprisingly not sex dependent. We show that syringeal muscle function has a high degree of thermal-dependence, as previously shown in insect superfast muscles, suggesting this is a common feature of superfast muscles. This thermal dependence has the potential to impact vocal behaviour in bats and birds, supported by reduced calling frequencies or switching to simpler call types with reduced body temperatures. Our in vitro measures were used to test novel computational muscle models, that opens new avenues of investigating muscle function in singing behaviour.

Poster Session 1 | Poster Wall 163 | Label: PS1.163

Category: Motor systems, sensorimotor integration, and behavior

Vocal exercise is necessary to maintain respiratory muscle performance in a songbird

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The production of complex learned vocalizations, such as human speech and birdsong, involves intricate and precisely coordinated movements executed by superfast muscles. We recently established that superfast vocal muscle required extensive use to achieve and maintain their adult performance. Short periods without singing drives changes in muscle performance with traces in acoustic output, such as changes in pitch and reduced source level, that females can detect and reject. We hypothesized that decreased source level is caused by decreased expiratory pressure resulting from weakened expiratory muscles. However, it remains unclear if respiratory muscles need motor practice to achieve and maintain performance, and whether such adaptations directly affect vocal output. Here, we test this hypothesis and measured intercostal muscle performance, in vivo expiratory pressure during singing in male zebra finches before and after 7-days of targeted singing prevention. One week of singing prevention approach led to significantly reduced source levels and reduced expiratory pressure. Expiratory muscle performance also changed: maximal isometric stress reduced, and maximal shortening velocities increased, consistent with the effects of exercise in limb muscle, but opposite to effects in syringeal muscle. Our data thus support the hypotheses that 1) expiratory muscles in songbirds require use to achieve and maintain performance 2) lower force results in lower expiratory pressure, which in turn 3) leads to lower source levels. Songbirds respiratory muscles thus weaken fast due to detraining, and could present an interesting novel model for weakened human respiratory muscles after periods on respiratory support in IC units. Additionally, these findings raise the fascinating question how the nervous system regulates this opposite effect of training on two motor systems that closely interact to produce vocal behaviors.

Poster Session 1 | Poster Wall 164 | Label: PS1.164

Category: Motor systems, sensorimotor integration, and behavior

Visuomotor control in virtually swimming Danionella larvae

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The ability to maintain a stable state in the presence of perturbations, known as robustness, is a defining feature of living systems. Most animals demonstrate adaptability by adjusting their position when confronted with external stimuli, a crucial skill for survival. How the brain dynamically regulates behavior in response to a changing environment remains a fundamental question in neuroscience. We investigated it using Danionella cerebrum, the smallest known vertebrate amenable to brain-wide functional imaging at cellular resolution across all developmental stages. We developed a 2D virtual reality system in which head-restrained larvae can navigate their visual environment. The system uses fluid dynamics estimates of the fish's intended movements to restore their expected visual feedback. We observed that they can continuously stabilize their position when subjected to external visual flows of varying speed and direction. We mathematically modeled this regulation process with a system of delay differential equations that can exhibit limit cycle oscillations, consistent with the observed speed fluctuations. Moreover, we were able to perform calcium imaging during these virtual reality experiments and identify neural populations spanning the entire brain with activities that correlate with specific features of both behavior and visual stimulation. Notably, we found assemblies of neurons that activate differentially during spontaneous or visually-evoked swimming. In conclusion, our study not only significantly advances our understanding of how animals integrate sensory input in real-time to drive motor actions, but also introduces analysis and modeling tools with broader applicability, which may prove useful to other researchers in the field.

Poster Session 1 | Poster Wall 165 | Label: PS1.165

Category: Olfaction, taste and chemical sensing

male silkmoth, Bombyx mori, in response to hierarchical

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The taste system controls many insect behaviors, but is greatly understudied in mosquitoes. Little is known about how tastants are encoded in mosquitoes or how they regulate critical behaviors. Here we examine how taste stimuli are encoded by the Aedes albopictus mosquito, a highly invasive disease vector, and how these cues influence biting, feeding, and egg laying. We find that neurons of the labellum, the major taste organ of the head, differentially encode a wide variety of human and other cues. We identify three functional classes of taste sensilla with an expansive coding capacity. Unexpectedly, in addition to excitatory responses we discover strikingly prevalent inhibitory responses, which are predictive of biting behavior. Certain bitter compounds suppress physiological and behavioral responses to sugar, suggesting their use as potent stop signals against appetitive cues. Complex cues, including human sweat, nectar, and egg-laying site water, elicit distinct response profiles from the neuronal repertoire. We identify key tastants on human skin and in sweat that synergistically promote biting behaviors. Our study sheds light on key features of the taste system that suggest new ways of manipulating chemosensory function and controlling mosquito vectors.

Poster Session 1 | Poster Wall 166 | Label: PS1.166

Category: Olfaction, taste and chemical sensing

Day/night cycles regulate pheromone acuity to gate rhythmic courtship behavior

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Day/night cycles profoundly impact animals' physiology and behavior to allow adaptation to rhythmic environmental cues. Daily rhythmic behaviors are believed to be patterned by central clock neurons. However, the physiology of primary sensory neurons, such as olfactory receptor neurons (ORNs), can also exhibit oscillatory changes, but the contribution of such peripheral neuromodulation to rhythmic behaviors remains undetermined. Here we showed that pheromone-sensing ORNs exhibit higher responses in flies at subjective night (henceforth referred to as night flies) than in flies at subjective day (henceforth referred to as day flies). Importantly, this heightened pheromone sensitivity in night flies in turn elevates odor-guided social behavior. Mechanistically, the day or night modulation is respectively signaled via two biogenic amines. Furthermore, the day/night modulation of olfactory acuity requires a cation channel subunit whose expression likely causes depolarization block to reduce ORN spike response frequency. As such, day/night cycles — through antagonistic actions of two biogenic amines — up- or down-regulate the cation channel in ORNs to dynamically modulate olfactory acuity and odor-guided behavior. Importantly, our findings highlight a critical role of peripheral sensory neuromodulation in gating rhythmic social behaviors.

Poster Session 1 | Poster Wall 167 | Label: PS1.167

Category: Olfaction, taste and chemical sensing

Harnessing the locust olfactory system for the detection of endometriosis

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Living systems have solved the problem of chemical sensing over millions of years of evolution and converged to a solution that is architecturally and functionally similar across species. This indicates that there might be an optimal solution for gas sensing that is still elusive from an engineering standpoint. Living organisms (e.g., canines) use biological olfaction to detect volatile organic compounds (VOC) mixtures via their robust odor recognition capabilities and generalization for chemicals across varying concentrations which can be harnessed for disease detection. Endometriosis is a gynecological disease characterized by the presence of endometrial tissue outside the uterus. Diagnosis of endometriosis is delayed an average of 7-10 years after the onset of symptoms due to the heterogeneity of symptoms and absence of specific biomarkers. Here, we address the challenge of detecting endometriosis via a biological olfaction-based sensor where we leverage the capability of the entire olfactory sensory system of an insect (locust) and analyze cell culture headspace-evoked neural responses to discriminate gas mixtures emitted from endometriotic vs healthy cells. Our results demonstrate that this approach can discriminate between multiple types of endometriotic and non-endometriotic cell lines. For this study, VOC mixtures emitted from the cell cultures were delivered to the locust antennae (biological chemosensory array) and the neuronal responses were obtained from the locust antennal lobe. Our analyses of neural responses show that individual neurons can distinguish each cell line by its ‘smell’ (i.e., emitted VOC mixture). By combining the neural responses across experiments, we obtained population neural response templates that were used to classify unknown gas mixtures to achieve high classification accuracy. This innovative insect-olfaction based disease detection approach leverages an entire biological olfactory system for the detection of endometriosis.

Poster Session 1 | Poster Wall 168 | Label: PS1.168

Category: Olfaction, taste and chemical sensing

Non-canonical encoding of human odor in mosquitoes

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Mosquitoes are animals that, by biting humans, transmit potentially deadly diseases. One prospective solution to this global health problem would be to control mosquito biting behavior. Female mosquitoes bite humans because they require a blood-meal to reproduce. To locate humans, mosquitoes rely heavily on their olfactory system. Human body odor is composed of a mixture of more than 500 odorant chemicals, many of which can vary between individuals. Despite this variation, mosquitoes are extremely successful at locating humans to bite. Mosquito olfactory sensory neurons have been recently described as deviating from the canonical motif seen in many olfactory systems in which each sensory neuron expresses only one type of olfactory receptor. Mosquito olfactory sensory neurons can co-express more than one type of olfactory receptor per sensory neuron. It remains unknown how this “non-canonical” olfactory receptor co-expression impacts odor detection by mosquitoes as they search for a human to bite. In this project I will use in vivo electrophysiology to characterize how olfactory receptor co-expression impacts the physiology and wiring of Aedes aegypti olfactory circuits, both at the periphery and in the central nervous system. The long-term goal of this project is to understand how odorants are detected and processed by the non-canonical mosquito olfactory system in order to ultimately identify evidence-based strategies to disrupt the detection of human odor and prevent mosquito biting behavior.

Poster Session 1 | Poster Wall 169 | Label: PS1.169

Category: Olfaction, taste and chemical sensing

Evolution of odorant receptors in a blood-feeding fly

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Tsetse (Glossina sp.) are hematophagous flies native to the tropical regions of sub-Saharan Africa. All tsetse feed exclusively on blood, but different species have specialised on different host animals. When blood-feeding, tsetse transmit trypanosomes, parasites that cause human and animal trypanosomiasis (sleeping sickness and nagana, respectively). To identify and home in on a host animal, tsetse use olfactory receptors to sense volatile odours emitted by the host.

An evolutionary analysis of the tsetse odorant receptor repertoire revealed that nine receptor families are expanded as compared to Drosophila melanogaster. Four of these are putative pheromone receptors. However, very little is known about volatile pheromones in tsetse, beyond a disputed larval pheromone and three recently discovered sex attractants. We therefore hypothesise that some of these putative pheromone receptors may have been repurposed for sensing host volatiles.

We address this hypothesis on multiple levels. First, we performed behavioural assays to confirm the role of the putative larval pheromone, showing that it has no behavioural relevance under naturalistic conditions. We then decided to focus on odorant receptor Or67d – the most dynamic receptor family with five copies. In contrast, D. melanogaster has only one copy: a highly selective pheromone receptor tuned to the volatile sex pheromone 11-cis-vaccenyl acetate (cVA). To our knowledge, cVA has no role in tsetse. To understand the evolution and function of Or67d, we present a new analysis of the evolutionary history of this receptor. We further characterised the receptors functionally, by cloning tsetse Or67d genes into the D. melanogaster empty neuron system and conducting single-sensillum recordings, stimulating with both putative pheromones and host odours. Our results not only shed light on the evolution and function of this tsetse olfactory receptor, but also have the potential to lead to improved vector control strategies.

Poster Session 1 | Poster Wall 170 | Label: PS1.170

Category: Olfaction, taste and chemical sensing

Olfactory control of visual preferences in the mosquito, Aedes aegytpi

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Multimodal integration shapes behavior in many organisms, and this is particularly true in mosquitoes. Aedes aegypti, the yellow-fever mosquito, uses multiple sensory inputs, such as temperature and odors, to locate its host. However, little is known about the visible spectra that attract mosquitoes, and whether congruent or incongruent odors modulate these preferences. We used a real-time 3D tracking system and wind tunnel to investigate the preference of female biting mosquitoes for different wavelengths of the visible light spectrum. We found that specific odors modulate the spectral preferences of certain wavelengths - human skin odor elicits an increased attraction to long wavelengths (orange and red to the human eye), whereas floral odors increase the attraction to the medium wavelengths (blue, green). Interestingly, long wavelengths dominate the human skin color spectrum, and filtering those wavelengths abolishes the observed attraction. Photoreceptor knock-outs targeting opsin1 and opsin2, or using the orco mutant, eliminated the attraction to visual objects. Finally, two-photon excitation microscopy in tethered mosquitoes confirmed that neuropil activity in the lobula, in the mosquito's optic lobe, is modulated by olfactory input. Collectively, our results show that specific odors modulate the mosquitoes' wavelength preferences and that the mosquito visual system is a promising target for inhibiting their attraction to human hosts.

Poster Session 1 | Poster Wall 171 | Label: PS1.171

Category: Olfaction, taste and chemical sensing

Decoding Neurogenetics of Olfactory Specialization in Blind Cavefish

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Organisms often display sensory specialization adapted to their environment, yet the precise genetic mechanisms driving specialization are largely unknown. We investigated this question in Astyanax mexicanus, a teleost fish species with two distinct ecomorphs, using quantitative behavioral analyses and quantitative genetics. The blind, dark-adapted cavefish morph displays heightened olfactory sensitivity and distinctive foraging behavioral strategies in response to low food odor concentrations, as compared to the river-dwelling, eyed surface morph. Previous research has also demonstrated differences between morphs in olfactory epithelium development and the proportion of olfactory sensory neuron subtypes.

We conducted a high-throughput behavioral assay to examine olfactory behavioral responses and sensitivity of 400 F2 cave x surface hybrid juvenile fish. We assessed individual animals' olfactory sensitivity over a four-day procedure, exposing fish to low to high concentrations of alanine. Using automatically tracked data, we measured changes in swimming behavior, including changes in mean position and speed upon odorant stimulation, and we manually assessed changes in basal swimming pattern. We used these metrics to compute olfactory detection behavior "scores" for each fish, revealing a continuum of olfactory capabilities in F2 animals. Animals ranged from those exhibiting minimal or no detection to all concentrations presented, to those demonstrating robust responses to the lowest odor concentrations.

We are conducting a quantitative trait loci (QTL) analysis with these animals to identify genomic loci and candidate genes involved in cavefish olfactory specialization. By integrating behavioral studies with quantitative genetics, our research aims to provide insights into the genetic architecture of sensory-driven behavior and uncover novel genes critical to olfactory processing and evolutionary specialization.

Support: FRM grant EQU202003010144

Poster Session 1 | Poster Wall 172 | Label: PS1.172

Category: Olfaction, taste and chemical sensing

Ant aggregation pheromones: from social behavior to neural coding

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The capacity to form groups is at the core of insect societies. However, how this ability is encoded in their sensory and neural systems is unknown. A pheromone can be expected to mediate aggregation in ants, similarly to other aggregative behaviors in insects, but this sensory cue has not yet been described in any ant species. Here, we provide evidence that there is a low-volatility odor cue that mediates nest aggregation in the clonal raider ant (Ooceraea biroi), present on both their cuticle and in their nests. We provide evidence that this cue is not a learned odor cue but a fixed (species-specific) cue that elicits nest formation across genetically distinct O. biroi populations. Furthermore, we show that this odor cue elicits nest-related behavioral tendencies. We describe our results to pinpoint the chemical basis of this aggregation signal. Finally, we describe our efforts to establish a contact-based calcium-imaging set-up to study how perception of this pheromone and related behaviors are encoded in olfactory sensory neurons, using an established transgenic line. Overall, O. biroi, with its genetic amenability and evidence of an aggregation pheromone, presents an exciting system to gain a mechanistic understanding of how sensory perception can sustain group formation and social behaviors in ants.

Poster Session 1 | Poster Wall 173 | Label: PS1.173

Category: Olfaction, taste and chemical sensing

Employing the honeybee olfactory system for detection of volatile human lung cancer biomarkers.

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Chemical sensing has been conserved throughout the animal kingdom, evolving highly specific and sensitive biological pathways to detect odors. In particular, insects such as honeybees exhibit sensitive and precise chemosensory abilities enabling detection of a variety of volatile chemicals at very low concentrations. Honeybee antennae are comprised of olfactory receptor neurons (ORNs) that send their outputs to the antennal lobe (AL), changing the spatiotemporal pattern of neural spiking in response to an odor, before relaying to higher-order centers. The combinatorial and spatiotemporal coding scheme displayed in the signaling pathways enables the identification of a large number of odorants and complex gas mixtures.

Volatile organic compounds (VOCs) are metabolic byproducts produced by pathological processes emitted in varying concentrations expelled through breath or other bodily fluids. Diseases and cancers can alter the emitted profile or fingerprint of these VOCs thus altering the low-level concentrations of the VOCs presented. Here, we examine the accuracy of the honeybee olfactory system in identifying low-level concentrations of human lung cancer VOCs. Odor concentrations of 1% (v/v), 0.01% (v/v), and 0.0001% (v/v) were used to resemble VOC levels of hexanal and methyl-heptane found in human exhaled breath. We employed in-vivo extracellular neural recordings from honeybee ALs using multi-channel electrodes to examine neural spiking responses throughout the odor presentation windows. Our results indicate that honeybees were able to successfully classify varying low-level VOC concentrations between the chemicals, demonstrating their ability to accurately detect and distinguish between cancer biomarker VOC levels found in breath. This study provides further insight into honeybee olfactory sensing and a framework for future detection of cancer VOC biomarkers present in exhaled human breath.

Poster Session 1 | Poster Wall 174 | Label: PS1.174

Category: Olfaction, taste and chemical sensing

Pleiotropy drives the functional coupling of the production and perception of mating signals

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Successful mating decisions are essential for the fitness of individuals. In many insect species, the decision with whom to mate depends on information communicated via cuticular pheromones, a complex blend of hydrocarbons and other organic molecules that are released by one animal to be sensed by another. Because mating pheromones must communicate precise and reliable information, the coupling between the production of specific pheromone molecules and their perception by the nervous system are expected to be relatively stable within species. Yet, surprisingly, previous studies have suggested that pheromones are remarkably flexible, and could rapidly evolve as species diversify. One possible explanation for this apparent puzzle is that some pleiotropic genes co-regulate the independent physiological processes responsible for pheromone production and perception. Subsequently, these genes could provide a path for the co-evolution of pheromone signals without losing the functional link between mating signals and their cognate receptors. The Ben-Shahar lab uses D. melanogaster and other Drosophila species to investigate the genetic and neuroethological mechanisms that drive mate choice behaviors and allow this rapidly evolving group of insects to maintain behavioral mating boundaries between closely related species.

Poster Session 1 | Poster Wall 175 | Label: PS1.175

Category: Olfaction, taste and chemical sensing

Towards a Partial Molecular Atlas for the Mosquito Olfactory System

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The highly organized structure of the olfactory circuit periphery provides an opportunity to understand the fundamental mechanisms behind specific behaviors and how they evolved. Because olfactory sensory neurons are functionally organized by the olfactory receptor proteins expressed, it is possible to uniquely express a transcription factor or other protein in just one olfactory neuron type. The systematic generation of transgenic lines expressing transcription factors in all of the various olfactory sensory neuron types creates a “molecular atlas” of the olfactory circuit periphery. A molecular atlas of the Drosophila melanogaster antennal lobe made it possible to dissect numerous olfactory mediated behaviors and gain deep insights into the neural circuits that generated these behaviors.

We are in the process of making a partial molecular atlas for the Yellow Fever Mosquito, Aedes aegypti. These CRISPR-Cas9 generated transgenic mosquito lines will allow us to investigate behaviors unique to the mosquito; moreover, they will allow us to understand the recent evolution of a preference for human odor in some populations of Ae. aegypti. Teasing apart the mechanistic basis for and the specific olfactory sensory neuron types in human odor preference will contribute to fundamental questions in neuroethology and evolution, and it will facilitate better mosquito control strategies.

This presentation will include a discussion of our strategy for selecting olfactory sensory neurons to target via single nucleus RNA sequencing data; insights from our present attempts to generate stable lines; and data from our first behavioral pilot experiments investigating the behavioral effects of silencing a single olfactory sensory neuron type.

Poster Session 1 | Poster Wall 176 | Label: PS1.176

Category: Olfaction, taste and chemical sensing

Identification of core genes of clock-controlled pheromone transduction in Manduca sexta

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Insect chemosensory transduction is still under debate. Mostly based upon studies in the fruit fly Drosophila melanogaster it is assumed that all insect olfactory receptor neurons (ORNs) employ ionotropic chemosensory transduction. But in the extremely sensitive male hawkmoths that can detect single pheromone molecules of their conspecific mates, we find no evidence for ionotropic pheromone transduction. Instead, with in vitro and in vivo electrophysiological analysis we find evidence for a G-protein coupled pheromone-transduction cascade involving phospholipase C activation. Furthermore, sensitivity and temporal resolution of pheromone transduction express daytime-dependent differences correlating with circadian changes in cAMP levels in hawkmoth antennae. To identify the molecular constituents and mechanism of hawkmoth pheromone transduction and its circadian modulation, we investigated dynamic transcriptomic changes in adult male hawkmoth antennae at different zeitgeber times (ZTs) comparing sleep- and activity phases of hawkmoths. We found daily changes in the expression of circadian clock genes period, timeless, clock, cycle, and cryptochrome. However, an array of potential participants in pheromone transduction, including 14 G-proteins, 254 ion channels, 43 participants in second messenger cascades, and 90 enzymes, exhibited no notable variance across different ZTs. We concluded that daily and circadian modulation of chemosensory transduction cascades is rather controlled via post-translational feedback loop (PTFL) clocks associated with the plasma membrane than by the transcriptional-translational feedback loop (TTFL) clock in the nucleus. To further challenge our hypothesis of metabotropic pheromone transduction under PTFL control and to identify signaling cascades present in single ORNs we employ single-nucleus RNA sequencing combined with quantitative PCR (qPCR), and physiological assays. [Supported by DFG grant GRK 2749/1 “multiscale clocks”]

Poster Session 1 | Poster Wall 177 | Label: PS1.177

Category: Olfaction, taste and chemical sensing

Ancestral complexity and constrained diversification of the and olfactory system

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Ants form a diverse group of eusocial species whose success likely relies on their heightened olfactory abilities. Typically, their group cohesion is mediated by odorous compounds, the cuticular hydrocarbons that form complex blends used as nestmate recognition cues but also in caste signalling and in mating context. In ants, the detection of these compounds occurs within specific structures on the antenna called basiconic sensilla. These basiconic sensilla house olfactory sensory neurons whose axons project within the brain into a distinct region of the primary olfactory neuropil, known as the antennal lobe, forming a specialized olfactory subsystem named the BaS (basiconic sensilla) subsystem. Considering the taxonomic and ecological diversity of ants, we conducted a comparative neuroanatomical study across fourteen species spanning the Formicidae phylogeny. Our study revealed that basiconic sensilla are mainly located in the distalmost segments of the antennae in all examined species. Additionally, our observations revealed the ubiquitous presence of the BaS subsystem within the antennal lobe of ants, showing consistent investment across lineages in terms of both volume and the number of glomeruli. Building upon our observations of extant species and the most recent phylogeny, we also assessed the evolutionary rates of olfactory features and reconstructed their ancestral states. Our model predicted the presence of a well-developed subsystem at the origin of ants, with a high number of glomeruli already present in their MRCA. Lastly, our findings suggest that the chemical complexity of species-specific cuticular hydrocarbon profiles is the most reliable predictor of olfactory evolutionary investment in the BaS subsystem.

Poster Session 1 | Poster Wall 178 | Label: PS1.178

Category: Olfaction, taste and chemical sensing

Synergistic olfactory processing for social plasticity in desert locusts

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Desert locust plagues threaten the food security of millions, as starvation compels this ancient pest to devour everything in its path. Central to their swarm formation is crowding-induced plasticity, with social phenotypes changing from cryptic (solitarious) to swarming (gregarious). Here, we elucidate the implications of this transition on foraging decisions and corresponding neural circuits. We used behavioral experiments and Bayesian modeling to decompose the multi-modal facets of foraging, revealing olfactory social cues as critical. To this end, we investigated how corresponding odors are encoded in the locust olfactory system, allowing the animals to make decisions appropriately and in a context-dependent manner. We established an in-vivo functional calcium imaging protocol to monitor the activity of antennal lobe projection neurons. Further, to characterize the spatiotemporal details of odor-induced responses, we developed a data-driven and reproducible approach for unsupervised activity-based segmentation that intends to overcome the challenges of traditional approaches. Through this, we discovered crowding-dependent synergistic interactions between the neural responses to food and social odors. The observed synergy was specific to the gregarious phase and manifested in distinct odor response motifs, which were distributed across stable combinatorial response maps. As a direct consequence, this allowed us to use the antennal lobe network dynamics to predict whether a locust was gregarious or solitarious reliably. Our results suggest a crowding-induced modulation of the locust olfactory system that enhances food detection in swarms. Overall, we demonstrate how linking sensory adaptations to behaviorally relevant tasks can improve our understanding of social modulation in non-model organisms.

Poster Session 1 | Poster Wall 179 | Label: PS1.179

Category: Olfaction, taste and chemical sensing

Mechanism of olfactory caregiver recognition by social tadpoles

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Across taxa, some of the very first social interactions involve communicating hunger and avoiding dangerous stimuli. It is vital for infants to be able to tell the difference between their caregivers and strangers and exhibit appropriate behavior in various social contexts. Despite the importance of the early stages of social cognition, our understanding of how the developing brain learns from sensory information to make context-appropriate decisions remains limited. To address this question, we use Ranitomeya imitator tadpoles which are excellent models of complex social behaviors as they display parental recognition and communication of their needs using a robust motor display (e.g. begging). R. imitator tadpoles rely on olfactory cues to recognize their caregivers. We show that exposure to different olfactory cues during development affects who R. imitator tadpoles differentially beg to. Tadpoles raised with exposure to sibling olfactory cues beg more to frogs of their own species as opposed to a conspecific species (Ranitomeya variabilis), who place their predatory tadpoles into R. imitator pools. However, tadpoles raised in isolation do not differentially beg to either species. To begin to understand the neural circuitry underlying this behavior, we perform in vivo multiphoton imaging of neuronal activity in R. imitator tadpole brains by bulk loading of calcium sensitive dyes into neurons. We also demonstrate labeling of the olfactory nerve by electroporating dextran-coupled dyes into the olfactory epithelium.

Poster Session 1 | Poster Wall 180 | Label: PS1.180

Category: Olfaction, taste and chemical sensing

Untangling Rhythms: Interactions at different timescales in olfactory receptor neurons in nocturnal insects

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Antennal chemosensory neurons play a crucial role in the survival of insect species. They allow detection and anticipation of social signals and of environmental changes. In hawkmoth, females emit pheromone pulses at ultradian frequencies at night to attract their conspecific mates, whereas in cockroaches the males are attracting female mating partners with pheromones.

We are studying molecular mechanisms that could enable pheromone-sensitive olfactory receptor neurons (ORNs) in the hawkmoth and the Madeira cockroach to entrain to multiscale oscillations in pheromone levels. Insect ORNs are endogenous circadian clocks that express daily/circadian rhythms in clock gene expression constituting a nuclear circadian clockwork based on transcriptional translational feedback loops (TTFLs). Currently, it is not known whether daily rhythms in sensitivity and temporal resolution of ORNs of or are under mandatory control of the TTFL-clockwork in the nucleus.

To study circadian control, we recorded action potential (AP) activity with long-term tip-recordings from pheromone-sensitive trichoid sensilla on the antennae of both insect species. Already in the absence of pheromone stimuli multiscale rhythms in spontaneous AP activity were observed. To determine whether/how both ultradian and circadian AP rhythms of ORNs are linked and whether they are controlled via the circadian TTFL clockwork we employed pharmacology and RNAi studies combined with electrophysiology and computational modelling. We found interlinked multiscale rhythmicity in ORNs´ spontaneous activity and searched for responsible pacemaker channels, focusing first on ORCO, the olfactory receptor coreceptor.

With these studies we attempt to decipher the ORNs´ multiscale clocks underlying temporal encoding.

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Poster Session 1 | Poster Wall 181 | Label: PS1.181

Category: Olfaction, taste and chemical sensing

Layers of complexity in the sensory systems of the nudibranch mollusc Berghia stephanieae

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Gastropod tentacles contain a diversity of receptors, including chemoreceptors, which they use for navigation to food and finding conspecifics to mate with. We investigated putative sensory neurons and their central projections in the nudibranch Berghia stephanieae using immunohistochemistry (IHC), in-situ hybridization chain reaction (HCR), and bulk nerve fills. We found peripheral neurons are distinguishable by neuropeptide expression and the expression of various ionotropic receptors, including the widely conserved chemosensory IR25a. Aminergic and peptidergic fibers, likely projecting from the brain, also innervate all pairs of sensory appendages. Projecting into the brain, there are stereotyped axon tracts, as well as glomerulus-like structures, regions of bilateral integration, and convergence of various sensory modalities to discrete neuropils. In several cases, nerve fills highlighted individually identifiable efferent neurons were noted as the source of innervation of the periphery. The presence of serotonin, histamine, and octopamine fields in specific areas of the brain is reminiscent of insect sensory neuroanatomy and suggests integrative processing. IHC labeling for PKA-Cα, a marker for arthropod mushroom bodies and learning and memory more generally, highlights several areas for further investigation. In summary, a diverse ensemble of peripheral neurons on different cephalic appendages projects to structured neuropils within the central brain of a nudibranch mollusc. Such complexity has implications for processing in this not-so-simple brain and likely shapes behavior extensively.

Poster Session 1 | Poster Wall 182 | Label: PS1.182

Category: Olfaction, taste and chemical sensing

A model investigation of synaptic transmission tuned via the Unc13 protein

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Short-term synaptic plasticity is a fundamental mechanism in neural computation, influencing sensory adaptation and working memory on timescales ranging from milliseconds to minutes. At the molecular level, the interplay between two processes of vesicle release establishes the depressing and facilitating components at a single presynaptic site. The composition of short-term plasticity is driven by the expression of the gene variants (M)Unc13A and (M)Unc13B, which are evolutionary conserved across invertebrate and vertebrate species. In this study, we introduce a modification of the well-established Tsodyks-Markram model for short-term plasticity, incorporating independent facilitating and depressing model components. Our model is constrained through in vivo intracellular recordings within the olfactory pathway of Drosophila melanogaster, enabling accurate reproduction of postsynaptic responses towards dynamic presynaptic stimulation patterns. With our refined model, we emphasize the importance of the interplay between (M)Unc13A- and (M)Unc13B-dominated synapses in fine-tuning transmission dynamics. Moreover, analysis of the tuned parameter sets allows for comparison between different knock-down experiments, providing direct biological interpretability of the model parameters. Our findings contribute to the understanding the molecular basis of short-term plasticity in olfactory processing. Further, our fitted model can be utilized in future studies to design neural circuit models covering a highly realistic representation of protein-dependent short-term plasticity.

Poster Session 1 | Poster Wall 183 | Label: PS1.183

Category: Olfaction, taste and chemical sensing

Circuit evolution mediating the environmental impact on Drosophila courtship

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Animals have evolved a vast diversity of behavioral strategies to ensure mating success in their natural habitats. Using Drosophila as a model, we investigated how the complex sensory environments in which courtship behaviors unfold contribute to behavioral evolution. Drosophila courtship takes place on patches of their host fruits, where many flies congregate. Replicating this environmental context, we found that sexual arousal in the host specialist D. erecta requires the presence of food volatiles, a dependence not observed in its generalist relatives. D. erecta males persistently court fly-sized visual targets in a virtual reality paradigm when food volatiles are present but are indifferent to the same visual cues in the absence of odor. Optogenetic activation of olfactory sensory neurons is sufficient to induce male courtship, while genetic silencing of olfactory signaling abolishes it, underscoring how the interpretation of visual cues depends on the perception of food volatiles in this species. Functional imaging reveals how the distinct environmental dependence on male arousal in D. erecta arises from the reweighting of olfactory inputs onto a conserved circuit architecture. We found that the sexually dimorphic circuitry that mediates male courtship in Drosophila is broadly activated by food odors in both D. erecta and D. melanogaster. However, a key distinction is that while D. melanogaster males persistently pursue visual targets even in the absence of food volatiles, these chemosensory cues gate and perpetuate male arousal in D. erecta due to the disinhibition of LC10a visual interneurons that mediate courtship pursuit. Thus, subtle evolutionary tinkering within an ancestral circuit architecture that integrates ambient chemosensory signals and male sexual arousal underlies the striking olfactory dependence of courtship in D. erecta.

Poster Session 1 | Poster Wall 184 | Label: PS1.184

Category: Olfaction, taste and chemical sensing

A comparative look at chemosensory brain regions across cephalopods with different lifestyles

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Squid and cuttlefish (collectively, decapodiform cephalopods) show highly variable lifestyles, with different combinations of nocturnal, diurnal or cathemeral, and benthic or pelagic life strategies. Distinct specializations in sensory systems and sensory processing are expected in association with these different habitats and lifestyles. While these species are widely recognized for their sophisticated visual systems, we hypothesize that benthic and nocturnal species will show enhanced reliance on chemosensory systems compared with diurnal/cathemeral and pelagic species. To examine this question, we used magnetic resonance imaging to examine the brain anatomy of three species of decapodiform cephalopods with different lifestyles, all collected from the same location (the shallow coastal waters of Moreton Bay, Queensland, Australia): Euprymna tasmanica (benthic, nocturnal), Sepia plangon (benthic, diurnal), and Sepioteuthis lessoniana (pelagic, cathemeral). Basic brain anatomy is largely similar across species, but differences are apparent in the relative size of structures that process visual stimuli (e.g., optic lobes) and chemosensory stimuli (e.g., inferior frontal lobe). Identifying differences in brain structure and connectivity across species will help us better understand how these animals process information and select appropriate behaviors, as well as how they have evolved and specialized to occupy different niches in the marine environment.

Poster Session 1 | Poster Wall 185 | Label: PS1.185

Category: Olfaction, taste and chemical sensing

Investigating the Co-option of a Pheromone Receptor as a Host Odor Sensor in Blood-feeding Flies

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Tsetse flies, under the genus of Glossina, are biting flies that inhabit much of tropical Africa and readily feed on the blood of humans, domestic and wild animals. Tsetse are the vectors of African trypanosomiasis, also known as sleeping sickness, which can be fatal to animals and humans without treatment. Different Glossina species have different host preferences and have adapted to different habitats–Glossina morsitans habituate widely in the East African savannah and mainly feed on cattle blood, whereas Glossina fuscipes is distributed across riverine woodland and mainly feed on human and lizard blood. Although it is established that tsetse use their olfaction to detect odors and identify potential hosts, there is little knowledge regarding the olfaction circuit that is responsible for the diverging host preferences. In the olfactory system, odorant receptors serve as chemoreceptors expressed in the cell membranes of olfactory receptor neurons, playing a pivotal role in detecting various odorants such as host volatiles. Certain odorant receptors exhibit high specificity, particularly those involved in crucial functions such as mating, responding to specific ligands. In both Glossina morsitans and Glossina fuscipes, five copies of the same gene were found to be homologous to Or67d of Drosophila melanogaster, a receptor known to regulate mating behavior by sensing the pheromone 11-cis-vaccenyl acetate. However, the function of this receptor in Glossina remains uncertain, and the ligand binding to this “orphan” receptor is unknown. Here, we aim to deorphanize this tsetse odorant receptor. We cloned tsetse Or67d into the Drosophila melanogaster empty neuron system and used single sensillum recording to deorphanize the odorant receptors. We used a panel of volatile host odors to find a best ligand for the different copies of Or67d. Our results offer plausible explanations for how different fly species adapt to diverse environments and preferences for specific hosts.

Poster Session 1 | Poster Wall 186 | Label: PS1.186

Category: Olfaction, taste and chemical sensing

Non-Canonical Olfaction in Disease-Vector Mosquitoes

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Mosquito-borne diseases affect millions of people worldwide and claim more than half a million lives each year. Mosquitoes rely heavily on human-derived chemosensory cues as they search for a blood meal. Understanding how mosquitoes detect and encode human odor would provide a major inroad to preventing mosquito biting behavior and disease transmission. The study of mosquito olfaction also provides an opportunity to address fundamental questions about chemosensory neuroscience in an organism whose behavior is driven strongly by their sense of smell. In the well-studied olfactory systems of flies and mice, individual sensory neurons express a single olfactory receptor type and project their axons to discrete regions, called glomeruli, in the antennal lobe or olfactory bulb, respectively. This “one-receptor-to-one-neuron-to-one-glomerulus” organization is believed to be a widespread motif in olfactory systems. We discovered that the olfactory system of Aedes aegypti mosquitoes has a radically different organization. There are many more olfactory receptors than antennal lobe glomeruli, and multiple chemosensory receptors are co-expressed within individual olfactory sensory neurons. This dramatic difference in sensory organization has wide-ranging implications for olfactory physiology in general and specifically the integration of human odor cues that support robust human host-seeking.

Poster Session 1 | Poster Wall 187 | Label: PS1.187

Category: Olfaction, taste and chemical sensing

Characterisation of a Hunger State-Dependant Switch in Olfactory Response Behavior

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Animals need to integrate several multisensory inputs, both synergistic and conflicting, to make appropriate decisions when navigating and foraging. These decisions are often modulated by changes in the animal's internal state, for example, when hungry, sleepy, or aroused. Investigating the functional connectivity between sensory and internal state representations will reveal the spatiotemporal dynamics of decision-making.

We investigated the mechanisms by which hunger modulates olfactory preference in Drosophila larvae. We have shown that hunger can switch odour aversion to attraction, where the hunger state information is mediated by a single descending serotonergic neuron, the CSDn, within the larval antennal lobe. Now, we investigate if this is a general phenomenon and if the CSDn also modulates odour preferences with an initially different fed valence. After starvation, attractive odours, such as Ethyl Acetate, become even more attractive and we show that this increased attraction also depends on serotonergic signalling. However, we hypothesise that potentially a different serotonergic subcircuit is required for this attractive odour modulation.

To investigate odour representation in the brain under fed and starved conditions, we utilise a microfluidics device to immobilise larvae for functional 2-photon imaging. This device also allows for an automated and temporally precise presentation of liquid olfactory cues.

Further, we are using our behavioural setup to screen for hunger state-dependent modulation of additional attractive and ecologically relevant odours. Unexpected preliminary results suggest that larvae show attraction towards an adult male pheromone already when fed.

Exploring a wider range of olfactory sensory cues will allow us to uncover different modulatory mechanisms and neural circuits underlying flexible decision-making in Drosophila larvae.

Poster Session 1 | Poster Wall 188 | Label: PS1.188

Category: Olfaction, taste and chemical sensing

A molecular approach to understanding the neuronal responses and interactions in maxillary palps of Aedes aegypti

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Aedes aegypti mosquitoes use a variety of cues to find hosts for blood feeding. These cues involve carbon dioxide (CO2), and host odors, which include many components such as lactic acid, 1-octen-3-ol, and ammonia. The maxillary palp is one of the two primary olfactory organs that detect odor molecules present in the environment. It comprises multiple capitate peg (cp) sensilla on its surface, and each sensillum houses three olfactory sensory neurons: cpA, cpB, and cpC. Notably, cpA is known to detect CO2 using a complex of Gr1 and Gr3 receptors, whereas cpC responds to 1-octen-3-ol using the Or8 receptor. However, the response spectrum of the third neuron, cpB, is poorly defined. Responses in these neurons are measured with extracellular single sensillum recordings, followed by spike sorting; however, variations and overlaps in spike amplitudes of different neurons can make it difficult to unambiguously identify the activated neurons from the recordings. To measure the responses of cpB neurons without confounding spikes from the other two neurons, we generated a double mutant line by suppressing the activity of the cpC neuron by knocking out Or8 using CRISPR-Cas9 in Gr3 mutants (which already has suppressed cpA activity). Using this line, we measured the odor-induced activity of cpB neurons using an extensive odor set comprising odors emitted by the host, oviposition cues, and repellants. We have recently shown that the spontaneous activity of cpA is reduced when cpC is activated, suggesting that these two neurons are ephaptically coupled. Here, we further used the Gr3 mutants, the Or8 mutants, and the double mutants to study the interaction dynamics among the cpA, cpB, and cpC neurons due to ephaptic coupling. The findings of our study contribute to the understanding of odor processing in the maxillary palps.

Poster Session 1 | Poster Wall 189 | Label: PS1.189

Category: Olfaction, taste and chemical sensing

The SoBee project – Unraveling the olfactory neuroethology of social immunity in honey bees

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The honey bee is a major pollinator for flowering plants worldwide and is thus critical for global agriculture. Within the last decades, bee colonies are on a drastic decline and some contributing factors are pesticides, climate change and diseases. Diseases play a prominent role in eusocial insects as they offer amenable conditions for their transmission. Thus, early detection of and an adequate response to pathogens is necessary for colonies to ensure their survival. Therefore, bees have evolved 'social immunity' referring to colony-level adaptations through collectively performed defenses to prevent pathogen infection. Such hygienic behaviors include uncapping and the removal of diseased brood/adults. Recent evidence suggests that social immunity is predominantly guided via olfactory signals emitted by the pathogen and/or the host. However, the molecular mechanisms driving social immunity remain to be elucidated. In the HFSP funded SoBee project we form a consortium to join expertise in entomology, synthetic biology and chemistry to identify causal factors underlying social immunity. We therefore search for pathogen and bee cues causal to recognizing infected sisters, by a) testing volatile organic compounds (VOCs) produced by infectious bacteria against honey bee olfactory receptors expressed in a microbial olfaction platform, b) performing metabolite profiling of honey bee glands and clonal isolates from metagenomic library screens to search for chemical cues correlating with social immunity and, c) testing VOCs in bioassays, bee brain activity and conduct hygienic behavioral studies on honey bees. In the Konstanz group, we use an automated Y-maze setup in controlled laboratory conditions to perform odor preference assays with different castes of bees as we hypothesize that hygienic behaviors are mostly performed by nurse bees. Once hygienic bees are identified, we perform calcium imaging to characterize the neural representation of those odors in the bee brain.

Poster Session 1 | Poster Wall 190 | Label: PS1.190

Category: Olfaction, taste and chemical sensing

Gustatory sensitivity to amino acids in bumblebees

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Pollinators can gather two types of food rewards from flowering plants: nectar, a sugar-rich solution, and pollen, tiny grains rich in protein and fat. Bees and other insects have taste organs on their mouthparts and antennae, which they use while visiting flowers to gather information about the nutritional contents of the food they are collecting. While it is known that bees can use the sugar concentration of nectar to inform foraging decisions, the role of taste in assessing pollen rewards is poorly understood.

To tackle this question, we recorded the extracellular activity of the four taste receptors within individual gustatory sensillae on the mouthparts of the buff-tailed bumblebee, Bombus terrestris. Each sensilla was exposed to aqueous solutions with increasing concentrations of either sucrose or one of four of amino acids (two essential, lysine and valine, and two non-essential, asparagine and glutamate). We quantified the concentration-dependent responses for the different substances tested and compared the responses between sucrose and amino acids, and between the different amino acids themselves. These experiments revealed that bumblebees have concentration-dependent responses to some of these amino acids and that their amino acid sensitivity is comparable to their response to sucrose.

These results provide important insights into the complexities of how bumblebees' gustatory sensillae respond to different tastants, with implications for the foraging tactics of bees. Our experiments show that bees are indeed capable of assessing the nutrients contained in both nectar and pollen, meaning they can, in principle, use taste to meet food demands when foraging. This can in turn help us understand how they select which flowers to forage from and which ones to ignore, a seemingly small choice which can however determine the reproductive success of both pollinators and flowering plants, including many important food crops.

Poster Session 1 | Poster Wall 191 | Label: PS1.191

Category: Olfaction, taste and chemical sensing

When Predation Becomes Escape: Quantify Mosquito Foraging to Understand Repellency

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Foraging is a fundamentally evaluative behavior; a foraging animal must appropriately integrate stimuli of opposite modalities into an adaptive strategy to obtain a meal. Female Aedes aegypti mosquitoes, vectors of several dangerous arboviruses, exhibit a robust host-seeking behavior to detect humans and obtain a blood meal. This behavior relies on the interplay of multimodal stimuli, some attractive and others aversive. In particular, insect repellents disrupt the mosquito's ability to detect and locate its prey, yet the underlying mechanisms remain unclear, with multiple hypotheses proposed. Additionally, how these repellent and attractive stimuli interact to shape the overall foraging strategy of mosquitoes remains to be established.

To address these questions, we have developed a custom-designed behavioral chamber, the mosquito HOSTel, which enables controlled and modular administration of volatile and contact chemosensory stimuli with opposing valences to freely moving mosquitoes. By integrating high-speed videography and state-of-the-art computer vision techniques, we conduct high-throughput quantitative assessments of host-seeking behavior. Additionally, we utilize machine learning-based software, such as SLEAP, to precisely track the trajectory and pose of individual mosquitoes. This approach's accuracy and precision allow us to disentangle the contributions of volatile and contact-based stimuli to the overall foraging phenotype. This behavioral quantification shows that avoidance dynamics are repellent-specific, with distinct repellents exhibiting distinct modes of action to keep mosquitoes at bay.

Our research aims to shed light on the intricate sensory mechanisms underlying host-seeking behavior in Aedes aegypti mosquitoes, with potential implications for vector control strategies and public health.

Poster Session 1 | Poster Wall 192 | Label: PS1.192

Category: Olfaction, taste and chemical sensing

Genetic and Neural Basis of Attraction of Gravid Aedes aegypti to African Bermuda Hay Infusions

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The mosquito Aedes aegypti is a prolific disease vector with explosive population dynamics. Its ability to thrive in urban environments lies in the ability of female Ae. aegypti to seek out standing pools of water to lay their eggs. Of note, females are highly attracted to the scent of decomposing botanical matter in water. Fermentative hay infusion preparations made from African Bermuda Grass (Cynodon nlemfuensis) are used to bait autocidal gravid ovitraps for Ae. aegypti surveillance and control in Puerto Rico. However, the molecular and cellular basis of mosquito attraction to this potent oviposition attractant is unknown. Using a two-choice wind tunnel assay, we tested Ae. aegypti mutants of olfactory co-receptor genes (Orco, Ir8a, Ir25a, Ir76b) and the CO2 receptor subunit gene (Gr3) to understand which of these chemoreceptors are necessary for female olfactory preference and attraction to hay infusion. While ionotropic co-receptor mutations did not yield a difference in preference for hay infusion over water, Gr3 and Orco mutations led to decreases in preference. For double mutants of Gr3 and Orco, preference for hay infusion was completely abolished. This suggests that both signaling pathways act additively to attract mosquitoes towards hay infusion and mediate olfactory choice. To determine the ligands mediating attractiveness of hay infusion, we are applying integrative mass spectrometry methods combined with behavioral testing of synthetic blends. Given a likely byproduct of anerobic fermentation from hay infusion is CO2, we are also performing behavioral and neurophysiological experiments to evaluate the role of this gas and CO2 receptor neurons in mosquito oviposition. Our data suggests a novel role for Orco(+) and Gr3(+) olfactory sensory neurons in mediating Ae. aegypti oviposition site search behavior and provides a new model system to explore how chemosensory circuitry is coopted across the mosquito lifecycle to support reproductive behaviors.

Poster Session 1 | Poster Wall 193 | Label: PS1.193

Category: Olfaction, taste and chemical sensing

Do bumblebees have preferred floral smells?

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Plant-pollinator interactions are intricate processes influenced by a variety of signals, including floral scents. Bumblebees are important pollinators, who encounter a vast array of flower odours, and can use them to make foraging decisions. However, the specific floral and olfactory preferences of bumblebees remain poorly understood.

This project aims to explore whether bumblebees exhibit preferences for distinct volatile compounds emitted by flowers and whether these preferences are bee species-specific. Bumblebees and floral volatiles were collected during the summers of 2022 and 2023 in Durham, UK. The collected volatiles underwent analysis using gas chromatography-mass spectrometry (GC-MS), identifying key compounds associated with flowers visited by seven sympatric bumblebee species. We observed that bumblebees have species-specific preferences for plant species. These preferences also appear to be sexually dimorphic. Patterns of potential floral smell preferences for the target species of bees are being identified. The next steps will involve evaluating bumblebees' behavioural responses to artificial floral blends.

This research is poised to offer crucial insights into European bumblebee species' floral and olfactory preferences, potentially influencing bumblebee conservation strategies.

Poster Session 1 | Poster Wall 194 | Label: PS1.194

Category: Olfaction, taste and chemical sensing

Experience-dependent plasticity of a highly specific olfactory circuit in Drosophila melanogaster

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Throughout its life, Drosophila melanogaster encounters a variety of odor cues that signal potentially harmful threats, which are detected by highly specialized olfactory circuits enabling the animal to avoid them. We investigated whether such crucial neural pathways are hardwired or can be modulated by olfactory experience. Using long-term exposure to geosmin, an indicator of potentially lethal microorganisms that is highly repellent to flies, we analyzed the anatomical, functional and behavioral consequences using state-of-the-art techniques. We demonstrate at the single cell level that the underlying neuronal circuitry in the antennal lobe undergoes structural changes leading to an increased volume of the geosmin-responsive glomerulus. In particular, two-photon microscopy combined with photoactivation of photoactivatable GFP in different neuron types and glia cells reveal that second-order neurons show neurite extension and synaptic remodeling after exposure, whereas olfactory sensory neurons and glia cells remain unaffected. These morphological changes are accompanied by changes in behavioral preference. Flies exposed to geosmin subsequently tolerate this innately aversive odorant in general choice and oviposition assays. Interestingly, the signal transduction in the antennal lobe remains largely unaltered, suggesting physiological effects in higher brain centers, such as the mushroom body or lateral horn. Our study shows that even a highly specific olfactory circuit is plastic and can adapt to new environmental conditions, which could contribute to the fly's ability to resist the effects of climate change and environmental pollution.

Poster Session 1 | Poster Wall 195 | Label: PS1.195

Category: Olfaction, taste and chemical sensing

Modulation of olfaction by multi-sensory integration and learning in Honeybees

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Honeybees are central place foragers collecting nectar and pollen for their colonies over long distances. For efficient foraging, it is essential to use different sensory modalities associated with flowers, mainly their colour and odour. So, associating the colour-odour combination with the presence of a reward might be a helpful strategy. We could show earlier that bees build cross-modal reward associations, allowing them to distinguish an olfactory visual compound from its single elements (light and odour). However, the neuronal basis for this multi-sensory integration remains to be determined. We have reasons to guess that mushroom body (MB) circuitry is involved in that computation for two reasons: (i) projection neurons of both modalities (olfaction and vision) innervate subpopulations of Kenyon Cells (KC). Out of these, around 180,000 KC, only about 400 MB output neurons (MBON) converge and probably combine this information. (ii) Individual MBONs can be recruited to encode the reward-associated stimulus. It is, therefore, conceivable that after building an olfactory-visual (compound) reward association, MBONs became recruited to code for the learned multimodal stimulus. Moreover, in a previous study focusing on naïve responses, we have shown that some subpopulations of MBONs were sensitive to only one tested modality (vision or light). In particular, more than 30% of the recorded MBONs responded to both modalities.

In the present study, we will use already established extracellular multi-unit recordings of MBONs in combination with olfactory visual compound conditioning and compare the neural activity before, during and after classical conditioning. We will compare the neural activity to the olfactory stimulus with the response induced by the same odour as a part of an olfactory-visual compound to check if the visual element might modulate odour perception after learning. We will also look at conditioned behaviour made by the proboscis extension response.

Poster Session 1 | Poster Wall 196 | Label: PS1.196

Category: Olfaction, taste and chemical sensing

State-dependent modulation of CO2 responses in the yellow fever mosquito Aedes aegypti

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Carbon dioxide (CO2) is a key chemosensory cue that is integrated alongside other physical and chemosensory stimuli to drive mosquito attraction to humans. It has been demonstrated that the behavioral responses exhibited by females of the yellow fever mosquito Aedes aegypti to CO2 stimulation is strongly influenced by their internal nutritional state, and that blood feeding suppresses behavioral activation seen in unfed mosquitoes. We have used the QF2-QUAS system to gain genetic access to olfactory sensory neurons (OSNs) expressing the CO2 receptor Gr1 to record odor-evoked responses of these OSNs in the maxillary palp. We found that by introgression of these transgenic reagents into a mutant background that lacks cuticle melanization the signal obtained from transcuticular imaging of sensory dendrites increased 4-fold. We additionally used these neurogenetic tools to record activity of MD1 glomerulus in the antennal lobe where CO2 sensitive OSNs project to. Furthermore, we assessed the influence of starvation and blood feeding in the response dynamics to CO2 in these sensory neurons. These optimized methods for peripheral and central imaging of mosquito CO2 olfactory responses have the potential to be applied to unravel the molecular mechanisms driving the nutritional-state dependent modulation of CO2 responses in mosquitoes by cell-type specific gene silencing.

Poster Session 1 | Poster Wall 197 | Label: PS1.197

Category: Olfaction, taste and chemical sensing

Odor generalisation versus discrimination driven by a connectivity bias for different ethological groups of odors in the Drosophila Mushroom Body

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The mushroom body (MB), the centre for olfactory associative learning in insect brain, performs pattern separation to discriminate between similar odors. We investigated mechanisms of odor discrimination in the Drosophila MB by combining connectome analysis, functional imaging, and network modelling. We found that the functional connections in MB deviates from the suggested optimum for odor discrimination and that different subtypes of MB neurons (MBNs) sample the olfactory space in distinctive ways.

MBNs receive combinatorial input from various types of olfactory projection neurons (PNs). In the presence of inhibition, MBNs exhibit sparse population coding. The connection from PNs to MBNs determines the MBNs' pattern separation capabilities: it is theoretically optimal when MBNs receive random inputs from PNs. Contrary to the common assumption that MBN subtypes receive similar information about odors for various memory processes, our analysis of multiple connectomes revealed that α/β and α'/β' MBNs receive highly biased inputs from food-odor-responsive PNs, while γ MBNs receive slightly biased inputs from mating-odor-responsive PNs. By recording functional activity at MBNs' soma, we showed that α/β and α'/β' MBNs form distinctly separated representations of food group odors and mating group odors. Additionally, within each odor group, odor representations are inherently clustered in the representational space of MBNs. By developing a MB network model that incorporates realistic PN-to-MBN-subtype connections, we showed that biased connection of α/β and α'/β' MBNs to food-odor-responsive PNs could increase their response overlap for food odors, when compared to random connections. Overall, these results suggest that the connections of α/β and α'/β' MBNs promote the generalisation of odors within the same ethological group such as feeding and mating, whereas γ MBNs, which do not show ethological groupings in their odor space, might enhance odor discrimination.

Poster Session 1 | Poster Wall 198 | Label: PS1.198

Category: Olfaction, taste and chemical sensing

Investigating brain anatomy across several bumblebee species, native to North-East England

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All over the world, bumblebees are declining in abundance. Their decline coincides with, and is likely a result of, increasing anthropogenic activities such as agricultural intensification, urbanization, and climate change [Nieto et al. 2014; Soroye et al. 2020]. Bumblebees are important pollinators for crops; hence, their continued decline could not only further harm environmental balance but might also disrupt worldwide food security [Khalifa et al. 2021]. Bumblebees Bombus terrestris and Bombus impatiens have historically been a subject of neuroethological and sensory neuroscience research [Mares et al. 2005; Li et al. 2017; Mertes et al. 2021], but relatively little knowledge has been acquired about other bumblebee species. We urgently need a better understanding of the biology and physiology of the Bombus genus, to aid conservation efforts, prevent further declines, and diversify their use in agriculture. Pollinators often use the volatile profiles of flowers to guide their foraging choices, and although bumblebees are often considered to be generalists when foraging, their plant preference is nevertheless species-specific (see [Sikora et al. 2020]). In addition, workers and drones of the same species often forage on different plants (see [McHugh et al. 2023]). In this project, I investigate whether these olfactory preferences are reflected in the anatomy of the olfactory brain centre – the antennal lobe. I focus on 7 bumblebee species native to Northeast England and, for the first time, compare brain anatomy across and within these bumblebee species through immunostaining, confocal imaging, and 3D reconstruction of their antennal lobes.

Poster Session 1 | Poster Wall 199 | Label: PS1.199

Category: Olfaction, taste and chemical sensing

Olfactory aversive conditioning and mixture perception in Drosophila melanogaster

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Odorants are detected by olfactory receptor neurons (ORNs) that project to the antennal lobe (AL), the first olfactory neuropil in the insect brain. In the AL, ORNs make synaptic contacts with: i) projection neurons (PNs), which in turn send olfactory information to other brain areas; and ii) local interneurons (LNs) that form a dense network of lateral inhibitory and excitatory interactions within the AL. Functional and computational studies indicate that this local network transforms sensory information, presumably to enhance perception of meaningful odor.

Here, we investigate the role of local GABAergic neurons both in relation to learning-dependent plasticity in the AL and the ability of flies to perceive the presence of learned odors in mixtures. For that aim, we performed aversive olfactory conditioning using a single odorant as conditioned stimulus and after that tested olfactory avoidance in a T-maze by exposing the flies to the conditioned odor pure or in different proportions mixed with a novel odor. We determined the threshold proportions that flies need to detect the learned odors immersed in the mixture. These proportions are odor and mixture specific. Next, we are studying whether blocking the activity of the LNs in the AL modifies the ability of the animals to detect learned odors embedded in mixtures.

Finally, we asked wheater olfactory aversive conditioning affects representation of odor mixtures in the antennal lobe. We recorded odor evoked responses of projection neurons using calcium imaging, while concomitantly training animals with the same protocol used in the T-maze. This protocol allows us to study odor representation before and after training. We found that the representation of a mixture in the AL changes after aversive learning.

Poster Session 1 | Poster Wall 200 | Label: PS1.200

Category: Olfaction, taste and chemical sensing

Behavioral, physiological and computational characterization of olfactory sensory adaptation

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The olfactory system is continuously exposed to an extraordinary range of chemical stimuli. To maintain sensitivity to meaningful odors the system must be adjusted based on animal's experience. One of the main phenomena that adjust the system is sensory adaptation, which is defined as a decrease in sensitivity or response to an odor after a sustained exposure to it and depends on the immediately close experience of the animal. In this project, we investigate the role and the mechanisms involved in olfactory sensory adaptation using honey bees. By performing electroantennograms we measured the activity of olfactory receptor neurons (ORNs) and characterized temporal aspects of this phenomenon such as induction, duration, and recovery time. We also analyzed whether adaptation depends on odor identity. We found that adaptation at the ORNs level, is odor specific and that odors that are relevant for the animal show a certain degree of resistance to adaptation. We also performed calcium imaging experiments to measure odor induced signals in the antennal lobe, the first olfactory neuropil in the insect brain. This allowed us to observe how adaptation changes the neural representation of odors and we describe that cross-adaptation occurs among odors that share activated glomeruli in the response pattern. Lastly, we developed a computational model of the olfactory system using an adaptive exponential integrate-and-fire model for ORNs and PNs. The model showed similar results to those obtained experimentally, indicating that a decrease in the response of ORNs and PNs is sufficient to observe sensory adaptation without the need for further plasticity. The results emphasize that sensory adaptation is critical to maintain the olfactory system unsaturated and ready to detect changes in the olfactory context.

Poster Session 1 | Poster Wall 201 | Label: PS1.201

Category: Olfaction, taste and chemical sensing

Diet, sociality, and taste evolution in ants

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Taste and sensory processing underpin food preferences, diet evolution, and species diversification. Ants have extraordinary species diversity and dietary variation (including live and scavenged invertebrate prey, extra-floral nectar, hemipteran honeydew, fruit, seeds, and fungus), making them excellent models for studying the role of diet and taste evolution in social organization and patterns of speciation. Insect taste receptors include gustatory receptors (GRs), ionotropic receptors (IRs), pickpocket (PPK) channels, and transient receptor potential (TRP) channels. While taste, or gustation, is relatively understudied in ants, recent effort from the Global Ant Genome Alliance (GAGA) to sequence 163 ant genomes has provided the opportunity to investigate taste and dietary evolution. Using annotated taste receptors from ant genomes, we inferred gene family phylogenies and the ancestral state for receptor numbers and dietary traits to gain insights into the evolutionary changes in diet and gene family repertories. In addition, we conducted phylogenetic least squares (PGLS) analyses to assess relationships between receptor numbers, and dietary, social, and ecological traits.Preliminary results suggest significant increases in GR numbers are driven by increased diet diversity and reveal GR subfamily-specific expansions and losses with dietary traits. These results, and similar results from other taste receptor families of interest, will provide new insight into the evolution of taste in a species-rich and ecologically diverse eusocial insect clade.

Poster Session 1 | Poster Wall 202 | Label: PS1.202

Category: Olfaction, taste and chemical sensing

Insight into the olfactory system of the migratory locust, Locusta migratoria: An anatomical and cellular study

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Olfactory cues are detected by odorant receptors in the olfactory sensory neurons (OSNs) located in hair-like structures called sensilla on sensory appendages like antennae and mouthparts. In most insects, the primary olfactory centre, the antennal lobe (AL), consists of distinct globular structures called glomeruli where OSNs, the olfactory output projection neurons (PNs) and the local interneurons (LNs) interact. Stereotypically, a one-to-one wiring logic occurs, where axonal projections of single OSNs and dendritic innervations of single PNs target predominately one glomerulus (uniglomerular OSNs/PNs). In contrast, locusts (Order: Orthoptera; Sub-order: Caelifera) exhibit a unique AL organization with over 2000 micro-glomeruli wired by multiglomerular OSNs and PNs, making locust an interesting case of the evolutionary study of olfaction systems.

Our study aims to thoroughly characterise the anatomical and cellular architecture of the olfactory system in Locusta migratoria. Specifically, OSNs of identified sensilla are traced using single sensillum anterograde staining in order to unravel their AL circuitry patterns, with respect to sensillum type (basiconic and trichoid) and topology. On the same grounds, focal injection and intracellular staining of PNs are employed for identification of PN types and their respective wiring from the AL to higher brain centres (mushroom body calyces and/or lateral horn). We believe that our study can provide insights into the complex construction of this particular olfactory system, contributing to a better understanding of its function.

Poster Session 1 | Poster Wall 203 | Label: PS1.203

Category: Olfaction, taste and chemical sensing

Evolution of the olfactory circuits driving human host preference in mosquitoes

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A recently derived subspecies of the mosquito Aedes aegypti has evolved to specialize in biting humans and is the primary vector for diseases like yellow fever, Zika, and dengue. In contrast, the ancestral subspecies resides in forests and bites a variety of animals opportunistically. Host preference is strongly influenced by volatile compounds emitted from the host's body. Host odors are complex blends with many shared compounds across vertebrate hosts, making it challenging to distinguish between them. How are these host odor blends encoded in the brains of mosquitoes with preferences for humans versus animals? And how does the evolution of this neural code reflect the evolution of their host preference?

To address these questions, we first assessed the importance of olfaction for host preference by comparing behavioral responses of both subspecies to animal and human odor, with and without a functional olfactory co-receptor orco. We also employed novel neurogenetic tools, odor delivery methods, and in vivo calcium imaging using the calcium indicator GCaMP to analyze neural responses to host odors in the antennal lobes of both subspecies. We observed notable changes in certain key olfactory glomeruli tuned to host odor compounds. Specifically, one glomerulus tuned to compounds more abundant in human body odor exhibited stronger responses in human-preferring mosquitoes. Conversely, another glomerulus tuned to compounds biased towards animals showed heightened responses in animal-preferring mosquitoes. The evolutionary shift from biting animals to humans might be closely linked to a simple rebalancing of odor sensitivity in these crucial information channels.

Our findings offer exciting new insights into the neural coding of ecologically relevant odor blends and the evolution of neural circuits that facilitate behavioral adaptations.

Poster Session 1 | Poster Wall 204 | Label: PS1.204

Category: Olfaction, taste and chemical sensing

Ephaptic coupling between olfactory receptor neurons is sensitive to relative stimulus timing: A potential mechanism for odor source discrimination

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Insect olfactory receptor neurons (ORNs) are often co-localized within sensilla and exhibit non-synaptic reciprocal inhibition through ephaptic coupling. It has been postulated that this inhibition aids odor source discrimination in turbulent environments, as synchronous arrival of different odorants from a single source should increase ephaptic inhibition, whereas asynchronous arrival of odorants from different sources should decrease ephaptic inhibition. Indeed, using behavioral experiments with free-flying flies, we previously showed that odorant onset asynchrony increases flies' attraction to a mixture of two odorants with opposing innate or learned valence.

However, the physiological underpinnings of such a millisecond timing based mechanism of source discrimination is not known. While ephaptic inhibition is a candidate mechanism, past studies have focused on ephaptic inhibition of sustained ORN responses to prolonged and constant odorant stimuli, begging the question whether temporal arrival patterns of different odorants indeed modulate ephaptic inhibition.

To investigate the role of ephaptic inhibition in olfaction within turbulent environments, we recorded co-localized ORNs in the fruit fly Drosophila melanogaster exposed to dynamic odorant mixtures. We found that ephaptic inhibition does indeed modulate transient ORN responses and is sensitive to the relative stimulus timing, with the strength of ephaptic inhibition decreasing as the synchrony and correlation between arriving odorants decreases.

These results show that ephaptic inhibition transforms the representation of temporally structured mixtures on the level of olfactory receptor neurons, which could form the basis of the behaviorally observed odor source discrimination based on temporal stimulus features.

Poster Session 1 | Poster Wall 205 | Label: PS.205

Category: Olfaction, taste and chemical sensing

An olfactory social language in the naked mole-rat?

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Olfaction plays a crucial role in the survival and social behavior of many species. One exceptionally social mammal is the naked mole-rat, which lives in large colonies led by one dominant female, known as the queen. She is the only breeding female capable of lactating in the colony. We hypothesized that despite its subterranean habitat, the naked mole-rat exhibits a keen olfactory sense which might foster social bonds within the colony and aid in identifying colony members, intruders, and potential threats. However, our understanding of the specific chemical cues governing their social and maternal behaviors remains limited. Here, we examined the chemical signals involved in the social communication of naked mole-rats and the underlying neurobiological substrates. Our chemical analyses of odor profiles from various members have unveiled the presence of a previously unknown queen-specific compound, which has also previously been detected in human breast odors. Electrophysiological recordings indicated that the queen odor is detected by the activation of olfactory sensory neurons in the main olfactory epithelium. Behavioral experiments indicated that females may exhibit attraction to this compound, while males display aversion. Furthermore, our findings reveal that different species of mole-rats exhibit distinct chemical profiles, with the naked mole-rat “queen” odor detected in social species, but absent in solitary species, highlighting the significance of this compound in social communication among African mole-rats. Our findings illuminate the role of olfactory communication in the social dynamics of naked mole-rats, providing valuable insights into the unique social structure and ecological niche of naked mole-rats.

Poster Session 1 | Poster Wall 206 | Label: PS.206

Category: Olfaction, taste and chemical sensing

Is age- and mating-dependent modulation of floral seeking in mosquitoes regulated by an odorant receptor switch?

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Female Aedes aegypti display dynamic resource-seeking behaviours, in bouts, during different stages of the adult life cycle. These behavioural bouts are modulated by internal physiology and external sensory stimuli, primarily olfactory cues. Teneral females are receptive to floral odours, owing to their innate requirement to locate sugar sources for energy. In contrast, older females develop the competence to take a blood meal and are attracted to vertebrate host cues. Although mating-induced modulation of host seeking has been observed in mosquitoes, the effect on nectar seeking has not been explored, nor has the mechanism for this switch been described. Previous antennal transcriptome analysis identified an overall and differential expression of odorant receptor genes, corresponding to the dynamic behaviour bouts, with rare exceptions, including odorant receptor Or117. We hypothesize that Or117 plays a role in regulating nectar seeking during adult maturation and following mating in Ae. aegypti. Using behavioural and electrophysiological approaches on mating-controlled teneral and mature females, we demonstrate a significant change in response to a synthetic odour of preferred floral sources. Mating in teneral females abolished the attraction, and altered the physiological response to the odour, while it had no effect in older females. Using a functional genomic approach, the ligand for Or117 was identified in the headspace of the most preferred floral source, Lantana camara. CRISPR-Cas9 knockout of Or117 or subtraction of its ligand from the odour, abolished the behavioural and physiological response of teneral females to the synthetic odour. Ongoing transcriptome analysis and multi-photon calcium imaging is aimed to further investigate the role of age and mating status on the differential gene expression at the periphery and differential processing of individual compounds in the synthetic odour at the level of the antennal lobe, respectively.

Poster Session 1 | Poster Wall 207 | Label: PS1.207

Category: Olfaction, taste and chemical sensing

Sense organs of Drosophila larvae

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Drosophila melanogaster larvae are a favored model organism to study principles of sensory perception. Of all senses, assessing food quality and innocuousness is one of the most crucial senses for survival, especially for Drosophila larvae but for all feeding species in general. Recent studies [1, 2] have described the larval sensory system on an ultrastructural level in detail, including the terminal organ, which is the major external taste organ in Drosophila larvae. However, the major internal taste or pharyngeal organs were only described either in publications from the 1980s, with limited ultrastructural detail [3] or without cellular resolution [4, 5]. To fill this gap, we analyzed the four major pharyngeal sense organs (or compound sensilla, respectively) on an ultrastructural level and used this knowledge to make well-grounded predictions about the function of their sensory neurons. These organs are the ventral pharyngeal sensilla (VPS), the dorsal pharyngeal sensilla (DPS), the dorsal pharyngeal organ (DPO) and the posterior pharyngeal sensilla (PPS). In addition, not all sensory structures are described and named in the pharyngeal region. Therefore, we aimed to identify all undescribed sensory neurons associated with the pharynx and to classify them according to their ultrastructure. A precise classification and nomenclature of the different types of sensilla across the entire larval body will be beneficial for future anatomical and functional studies.

Poster Session 1 | Poster Wall 208 | Label: PS1.208

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Wings to Waves: The remarkable hearing of Anopheles gambiae

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For the males of the malaria vector Anopheles gambiae, the sounds of flying females are crucial cues for finding their mating partners. Male Anophelines are, however, incapable of hearing their conspecific females' flight tones directly. Their hearing is distortion-based, and relies on distortions produced by the mixing, in their ear, of their own and the females' flight tones.

Notably, the distortions produced in the male ear are orders of magnitude smaller than the flight tones themselves. Our data suggest, however, that the size of the distortion is not indicative of its importance. We stimulated male Anopheles gambiae ears with pairs of tones: One tone simulated their own flight tone, and the other was meant to simulate an approaching female. We subsequently probed distortion generation mechanically, via Laser-Doppler-Vibrometry of their flagellar receiver, and electrophysiologically, through extracellular recordings of local field potentials. We show that distortions corresponding to as small as ~10nm displacements (at the flagellar tip) evoked substantial nerve responses.

Production of these audible distortions in the male ear depends on two factors: The mosquitoes must be beating their wings at within the right frequency ranges, and the two flight tones must arrive at the male ear with sufficient energy. How mosquitoes are able to cope with these auditory demands aerodynamically is however not known. We show here that the aerodynamic forces produced by mosquitoes flapping at different wingbeat frequencies are mechanistically the same; mosquitoes are able to balance force production while changing their wingbeat frequency by modifying different wing kinematic parameters. Finally, we show that the soundscapes of both males and females are reminiscent of acoustic dipoles. With this information, and in conjunction with the two-tone experiments, we were also able to estimate the space around a male mosquito over which females can be expected to be audible.

Poster Session 1 | Poster Wall 209 | Label: PS1.209

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Analogous computational principles of visual and mechanical looming threats?

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Attacking predators produce danger-evoking visual and mechanosensory cues, and the prey escape success depends on its ability to accurately react to the threat on time. When a predator approaches at a constant speed, it produces on the visual field a spot whose angular size (a function of the ratio between the predator size and velocity) increases non-linearly with time. In a wide range of invertebrates, η neurons (angular size threshold neurons) have been identified.

In contrast to visual cues, the neural basis of the computation of mechanosensory looming has received much less attention. Both visual and mechanosensory looms are characterised by a non-linear acceleration of a relevant variable (angular size, wind velocity) and depends on the approaching object size (l) and velocity (v). Given this similarity and the selective pressures on evasion systems, we addressed the following questions using crickets as model systems (i) to which of the two parameters of the looming crickets would be sensitive? (ii) is there any evidence for η-like mechanosensory looming sensitive neurons?

After characterizing the aerodynamic signature of our stimuli with computational fluid methods, we stimulated crickets with translating spheres of various sizes and velocities to mimic predator attacks. Behavioural experiments showed that large and fast spheres are better detected than small and slow ones. Intracellular recordings of giant wind-sensitive interneurons supported the behavioural observations concerning the effects of predator size and velocity on predator perception. Moreover, we recorded neurons whose peak firing rate time occurs at a fixed constant flow velocity after a fixed delay δ, analogous to η neurons involved in visual looming detection. Finally, we implemented a computational recurrent-spiking model of predator perception in the cricket which includes our experimental observations. The implications of our model in terms of neuromorphic computing will be discussed.

Poster Session 1 | Poster Wall 210 | Label: PS1.210

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Navigating complexity: understanding rheotaxis in Ciona larvae

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Protochordates, such as Ciona, are often regarded as the simplest structured chordates, possessing highly streamlined nervous systems inclusive of sensory organs. As the sister group to vertebrates, they offer valuable insights into the sensory and neurophysiological mechanisms of chordate ancestors. Additionally, Cionas, or sea squirts, exemplify a typical biphasic planktonic life cycle where navigation of the larvae in the complex marine environment plays a crucial role and serves as a model for planktonic studies.

Our study investigates the behavior of swimming Ciona larvae under naturalistic conditions, specifically focusing on their response to water flow and grouping context. By employing deep learning tools for pose estimation, in conjunction with microfluidics and calcium imaging techniques, we scrutinized the rheotactic behavior of larvae and their sensory processing. Our findings reveal pronounced positive rheotaxis in Ciona larvae, with notable behavioral alterations observed in response to changes in group density. Removal of sensory cilia from epidermal sensory neurons diminishes the larvae's rheotactic ability. We suggest that early chordates possessed cellular and behavioral correlates of rheotaxis similar to those found in vertebrates. Furthermore, we propose a hypothesis regarding the neural circuitry that potentially underlies rheotaxis. By focusing on the mechanisms of sensory processing in a simple chordate, our research offers insights into the evolutionary facets of rheotaxis in vertebrates.

Poster Session 1 | Poster Wall 211 | Label: PS1.211

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Mechanosensory encoding by Johnston's organs in the antennae of hawkmoths

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The Johnston's organ (JO), located at the passive pedicel-flagellum joint of the antenna of insects, is a functionally diverse mechanosensory organ. It mediates various functions ranging from audition, tactile sensing, and airflow detection to various flight-related reflexes in diverse insects. In hawkmoths, the JO provides crucial vestibular feedback that ensures flight stability. Intracellular characterization of the JO neurons shows that they encode frequencies in a range-fractionated manner, which explains their ability to be extremely sensitive while also covering a large range of mechanosensory stimuli that enable their multifunctionality. It is not clear, however, which specific features of the stimuli are specifically encoded by the JO. To address this question, we proposed the hypothesis that the JO neurons encode the angular positions and velocities of the flagellum. We recorded intracellularly from the axons of JO neurons while moving the flagellum by a fixed angle at various velocities, and by various angles at fixed velocities. These experiments demonstrate that JO neurons encode one or a combination of stimulus features such as angular position, angular velocity, onset/offset and direction of flagellar movement. Drawing parallels with studies on the femoral chordotonal organs from which the JO may have evolved, we show that the encoding properties of JO resemble those of the femoral chordotonal organs and the campaniform sensilla of insect legs, the coxo-basal chordotonal organs in crab legs, and the intraspinal mechanosensory neurons in lampreys. We also compare and contrast our characterization of the neurons based on specific hypotheses to that obtained using a systems-level analysis such as white-noise analysis in which the temporal context of the stimulus may be scrambled. Our study shows that these two methods can sometimes yield very different results, which throws light on the constraints and limitations of the white noise analysis.

Poster Session 1 | Poster Wall 212 | Label: PS1.212

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Seeking amongst the clusters: a comparative transcriptomic study of the hygro- and thermosensory neurons in D.melanogaster and Aedes aegypti

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Humidity and temperature are omnipresent environmental factors that influence fitness, reproductive behaviour and the geographic distribution of terrestrial animals.

Unfavourable combinations of temperature and humidity (e.g. high temperature and either very high or very low humidity) are increasing the danger of overheating or dehydration. At lower temperatures, poikilotherm animals show a higher sensitivity to humidity levels than homeothermic animals. Due to their small size and therefore lower storage capacity for water, insects are especially at risk of desiccation making them an interesting model to study humidity and temperature sensing behaviour.

Furthermore, specific neurons for humidity sensing, the hygrosensory receptor neurons (HRNs), have been described and studied in a wide variety of insects. While humidity and temperature are environmental cues, common disease-vectors like the yellow fever mosquito (Aedes aegypti) and the tsetse fly (Glossina pallipides) also rely on humidity and temperature cues to find their host and egg-laying sites.

In D. melanogaster and Aedes aegypti specific neurons for humidity (hygrosensory receptor neurons, HRNs) and temperature (thermosensory receptor neurons, TRNs) sensing are located on the antenna.

Hygrosensation is driven by a triad of neurons: a moist cell, a dry cell and a hygroool cell. Additionally, the antenna houses hot and cold cells.

By conducting a comparative comprehensive transcriptomic analysis of these neuronal groups, we aim to uncover the key features of the neuronal mechanisms mediating hygro- and thermosensation in the insect antenna. Our findings reveal distinct gene expression patterns associated with the five different types of neurons (moist, dry, hygrocool, hot and cold). There findings provide valuable insight into the neuronal mechanisms of humdity and temperature sensing behaviour.

Poster Session 1 | Poster Wall 213 | Label: PS1.213

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Motion detection in the infrared realm: how ball pythons respond to target motion in predator-prey scenarios

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Infrared (IR) sensation allows for the detection of objects based on the amount of radiant heat they produce. Among vertebrates, three families of snakes have independently evolved IR sensitivity: boas, pythons, and crotalines. Snakes use IR sensing primarily in prey capture, although its use in nesting and thermoregulation has also been proposed. Previous research, mostly in crotalines, has shown that the IR system allows snakes to precisely strike warm objects in the absence of any other sensory input. Such spatial accuracy is supported by processing in the intermediate layers of the optic tectum (OT) that generate an egocentric map of the IR realm surrounding the animal. However, positional information alone is often not enough to guide predatory or navigation behaviors, as in nature IR-emitting prey animals frequently move. We are using juvenile ball pythons (Python regius) as a model system to understand whether and how motion is used by the IR system to guide predatory behavior. Using an electrically controlled heating device mounted on a XYZ-gantry system, we can control moving IR stimuli similar to those a snake would encounter in a natural hunting scenario to examine responses to different types of motion. These results will allow us to determine some of the capabilities and limitations of the IR system of pythons, as well as to generate hypothesis regarding the function of IR responsive circuits that we will test through electrophysiological recordings from tectal neurons. To facilitate these experiments, we are using standard light microscopy of fixed sections of the OT to determine the best approach to position electrodes in the IR-dedicated layers deep in the OT. This work is the first step in a series of experiments that aim to understand the neural processes underlying integration of visual and IR stimuli, and shed light on the mechanisms that allow the construction of coherent representations of the world when multiple sensory inputs are involved.

Poster Session 1 | Poster Wall 214 | Label: PS1.214

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Mechanosensory representation of wing deformations

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Dragonflies are well known for their spectacular aerial maneuvers. Their large flexible wings have practically infinite degrees of freedom and dynamic states, so substantial sensory feedback is required to represent the wing deformations that occur during flight. Using electrophysiological, behavioral, and computational techniques, we describe the wing state during natural behaviors and identify receptive fields and encoding properties of a class of wing mechanosensors. To extract the dominant features of wing motion, we digitized wing deformation using high-speed video of fixed-wing preparations as well as free flight behavior. We then used dimension reduction techniques to characterize the wing state and found that 99% of the wing's natural variability can be described with three parameters - bend, twist, & camber. Modal analysis of our high-fidelity finite element model also validated these primary structural modes. To test whether the deformation features were represented in the sensory signal, we recorded extracellularly from wing nerve afferents in response to wing flutter driven by airflow or forced vibrations. Surprisingly, turbulent air induces relatively stable wing oscillations and while there are hundreds of sensors distributed across the wing surface, a few large units dominate recorded signals. These sensors are phase-locked to wing oscillations and their timing is linked with deformation amplitude. In contrast, forced vibrations result in unnatural patterns of wing deformation, increased spike frequency, and recruitment of additional units. Combined with our neuroanatomical maps and ablation experiments, the electrophysiological results indicate that wing deformation sensors function as perturbation detectors. A few units continuously monitor natural deformations via phase information, and additional units are recruited to encode unexpected wing states, allowing dragonflies to closely monitor wing deformations during demanding maneuvers.

Poster Session 1 | Poster Wall 215 | Label: PS1.215

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

The anterior lateral line contributes to prey detection in larval zebrafish

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Natural environments are filled with chaotic and potentially discordant sensory stimuli, and a central task of the brain is to extract coherent information from these multisensory inputs. As larval zebrafish begin to hunt, they likely exploit all available sensory channels for detecting and localizing prey. The vast majority of studies on larval zebrafish prey capture behavior have thus far only examined the contributions of vision, identifying retinal, pretectal, and tectal circuits required for hunting behavior. Although visual information is sufficient in eliciting prey capture, behavioral evidence from a range of fish species suggests that the lateral line, a mechanosensory system that enables the animal to detect hydrodynamic stimuli, may be involved as well. Most of these studies, however, were done in juvenile or adult fish. Here, we tested the possible contributions of the lateral line to larval prey capture. Removing lateral line sensory inputs via laser ablation of the anterior lateral line nerve caused a decrease in hunting-associated eye convergence and reduced prey capture success in free-swimming larvae. Conversely, optogenetic activation of anterior lateral line neuromasts triggered eye convergences and J-turns, both behavioral hallmarks of larval prey capture. Lastly, by comparing the expression of cfos in blind and sighted fish following hunting behavior, we found differentially activated brain regions, including the optic tectum, medial octavolateral nucleus, and the habenula. We hope that our findings will open new avenues for ethologically-relevant studies of prey capture behavior and afford an opportunity to study multisensory integration in a genetically tractable organism.

Poster Session 1 | Poster Wall 216 | Label: PS1.216

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Life with long appendages: functional, kinetic and morphological adaptations in house centipedes

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The house centipede, Scutigera coleoptrata, is known as one of the fastest invertebrate predators. Although not too welcome inside homes, these centipedes are regarded as excellent biological pest control around vineyards. Endemic to the mediterranean region, this species is nowadays also common throughout central Europe. Scutigeromorph centipedes are generally characterized by extremely long legs and antennae, covered with thousands of sensory structures. They are exceptionally fast and agile, and when hunting, they use their slender legs like lassos to immobilize prey before injecting their venom. Most outstanding, and unique to centipedes, is the functional transformation of the last pair of legs, called the ultimate legs. They are not involved in locomotion or prey capture, are longer and have a higher number of sensory structures than normal locomotory legs. Moreover, their position and kinematics greatly resemble those of the antennae. Thus, it is assumed that they have a predominantly sensory function. Focusing on mechanoreception and olfaction, we aim to better understand the neurological and functional aspects of scutigeromorph appendages, using the house centipede as a model. By means of electrophysiology, antennal and leg nerve backfills, as well as immunohistochemistry and morphological investigations we want to determine to which extent the ultimate legs act as “posterior antennae”, and how the structure of the corresponding ultimate leg ganglion correlates to this function. Furthermore, we aim to understand the kinematics behind their fast locomotion. High-speed video recordings, as well as step-force measurements will reveal the locomotory mechanism they evolved in order to achieve their exceptional speed and predatorial success.

Poster Session 1 | Poster Wall 217 | Label: PS1.217

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

How flies and vector mosquitoes sense heat and humidity (and you)

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Temperature and humidity are environmental variables with major effects on insect survival and reproduction. Temperature affects all aspects of physiology, and animals rely on thermosensation to maintain body temperatures compatible with survival. Humidity is also significant for insect physiology. Insects are prone to dehydration and rely on humidity sensation (hygrosensation) to maintain appropriate hydration. In addition to such homeostatic roles, temperature and humidity are used by vector mosquitoes (which spread malaria, dengue, and other diseases) to help locate and bite humans. Humidity sensation also helps female mosquitoes locate water to lay eggs, as their larvae are aquatic. Thus, identifying and characterizing the molecular and cellular mechanisms by which insects sense and respond to temperature and humidity has potential relevance for understanding insect physiology and the transmission of disease.

In work in Drosophila, we (and others) have identified multiple molecular receptors that mediate these responses. These include the Transient Receptor Potential (TRP) channel TrpA1, the Gustatory Receptor Gr28bD, and several Ionotropic Receptors (IRs), including Ir21a, Ir25a, Ir40a, Ir68a and Ir93a. We will present new work in Drosophila focusing on the identification of previously unappreciated classes of insect thermosensory neurons and the mechanisms that underlie their thermosensitivity.
In addition, we have found that IR family members initially studied in Drosophila also have key roles in heat and humidity sensing in the malaria vector Anopheles gambiae and the arbovirus vector Aedes aegypti.
Here we present new work that extends these studies, establishing how different IR family members contribute to host seeking, blood feeding and oviposition site seeking. Together, these IRs, and the neurons that express them, combine to allow mosquitoes to help perform behaviors critical for disease transmission and mosquito reproduction.

Poster Session 1 | Poster Wall 218 | Label: PS1.218

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Myosin II actively regulates Drosophila proprioceptors

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Auditory receptors can be motile to actively amplify their mechanical input. Here we describe a new and different type of motility shaping physiological response of mechanoreceptors that resides in support cells. In Drosophila larvae, supporting cap cells transmit mechanical stimuli to proprioceptive chordotonal organs. We here report that the cap cells are strongly pre-stretched at rest to twice their relaxed length. The tension in these cells is modulated by non-muscle myosin-II motors. Activating the motors optogenetically causes contractions of the cap cells. Cap-cell-specific knockdown of the regulatory light chain of myosin-II alters mechanically invoked receptor responses, converting them from phasic to more tonic, impairing mechanosensory adaptation. This result suggests that two motile mechanisms might operate in concert in insect chordotonal organs, one directly in the sensory receptor neurons, based on dynein, and the other in the support cells, based on myosin.

Poster Session 1 | Poster Wall 219 | Label: PS1.219

Category: Other sensory systems (mechanosensation, anemosensation, thermoreception, hygroreception and nociception)

Magnetoreception in Cataglyphis ants

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The geomagnetic field offers useful cues for navigation, especially for long-distance migrants such as birds, sea turtles, lobsters, and butterflies. While magnetoreception has been studied intensively, the underlying mechanisms remain elusive. A crucial question for a mechanistic understanding of magnetoreception is whether animals rely on inclination- or polarity-based magnetic information. Cataglyphis ants are ideal experimental models for insect navigation and magnetoreception. They are solitary central-places foragers with excellent navigational skills. When the ants leave their underground nest for the first time to become foragers, they perform learning walks for up to three days to learn the visual panorama and calibrate their compass systems. The ants repeatedly stop their forward movement during learning walks for performing turns (pirouettes) interrupted by stopping phases. The gaze direction during the longest stopping phase is usually directed towards the nest entrance. During learning walks, ants rely on the geomagnetic field as a reference system and not on celestial cues to align their gaze direction. We could show that both a rotating UV polarization pattern and natural geomagnetic field conditions during learning walks are essential to induce learning-dependent neuronal plasticity in high order brain centers such as the mushroom bodies (centers for learning and memory) and the central complex (center for navigation). Cataglyphis' magnetic compass is a unique example of an essential magnetic compass used for close-range navigation, and may have a biological importance equal to that of animal species that pursue different navigational tasks like migration over long distances. We hypothesize that Cataglyphis' magnetic compass is polarity-sensitive, light-independent, and magnetic-particle based, and that magnetoreception is an active-sensing process involving sensory capacities of the ants' antennae.

Poster Session 1 | Poster Wall 220 | Label: PS1.220

Category: Vision and photoreception

Implications of Quantitative Visual Modelling

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Our understanding of the adaptive nature of correlations between visual information gained from (or obscured by) animal colouration and predator or conspecific behaviour is shaped by the tools available for their assessment. However, methods for quantifying the design and function of animal colouration have developed and diversified continuously, especially considering approaches using mathematical approximations of animal vision. Contemporary methods contrast with the past and enable the capture of colour patterns in various quantitatively and qualitatively sophisticated ways. However, few approaches to colour pattern quantification have been tested in the perceptual contexts in which researchers use them, challenging the forming and testing of hypotheses on animal colouration based on generalisable assumptions amid a growing palette of available tools. How can we navigate the growing diversity of tools and methods available for studying animal colouration through the eyes of ecologically relevant observers? How should researchers choose among a multitude of colour pattern measurements that are equally – yet only hypothetically – meaningful in one context but not in another? I aim to highlight recent studies which exemplify the implications of applying visual modelling at quantitative scales and discuss possible approaches to the opportunities and challenges of high-dimensional datasets in visual ecology.

Poster Session 1 | Poster Wall 221 | Label: PS1.221

Category: Vision and photoreception

Why the comb jellyfish Mnemiopsis leidyi flashes at night

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Bioluminescence is a fascinating trait found in the majority of marine animals and in the deep sea and in the oceans in general at night it is a dominating visual stimulus. Still, there is surprisingly little knowledge about the functionality of the light emissions. Theoretically, the functions fall in three categories; aid in prey capture, defense mechanism against predators, and intraspecific communication but there are very few experimental studies actually placing specific bioluminescent events in either category. Some of the most brightly bioluminescent animals are ctenophores, comb jellyfish, incl. the highly invasive species Mnemiopsis leidyi. They have photophores in rows following the ctenes that emit strong blue-green flashes, which can be triggered by mechanical stimulations. Their naturally occurring bioluminescence is little studied but an anti-predatory startling effect is a frequently proposed hypothesis for the function of the light emissions in comb jellyfish in general.

Here we have tested the bioluminescence of M. leidyi under semi-natural condition. We found that they produce spontaneous flashes when undisturbed and that the flash frequency increased in the presence of potential prey items. These spontaneous flashes were of short duration and produced by small areas of photophores. Furthermore, we found that collision with known predators from Danish waters (the moon jellyfish and 3-spined stickleback) produced larger and longer lasting flashes. Combined our results suggests at least two none mutually exclusive functions of bioluminescence in M. leidyi; a prey lure and a so-called burglar alarm used to attract secondary predators. We found no evidence of a startling effect.

Poster Session 1 | Poster Wall 222 | Label: PS1.222

Category: Vision and photoreception

An AAV based strategy towards functional 2P imaging in avian retina

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Birds have some of the most high-performing and anatomically complex eyes among all vertebrates, however, direct insights into the inner workings of their retinas remain limited and poorly understood. This is partly because of a lack of tools for expressing genetically encoded biosensors in avian retinal neurons. Here, we present our ongoing progress in overcoming this major technological challenge. Specifically, we use a custom-made ocular injection apparatus to administer diverse adeno-associated virus (AAV) serotypes into the retina of birds. Depending on the virus and its inherent retinal tropism, this technique should differentially target the outer and inner retinal neurons of the avian retina, including subsets of photoreceptors, horizontal cells, bipolar cells, Mueller glia, etc. Based on the preliminary success of this ocular injection technique in passerines using GFP reporters, we are now working on transferring the knowledge and procedures for expressing calcium and glutamate biosensors in chicken and zebrafinches, with the eventual goal of imaging light-driven activity under 2-photon microscopy, using working protocols established on other non-avian animals such as zebrafish, frogs and mice.

Poster Session 1 | Poster Wall 223 | Label: PS2.223

Category: Vision and photoreception

Revealing the hierarchical organization of the cephalopod optic lobe

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The cephalopod optic lobe (OL) is independently evolved from, and appears to be organized radically different to, the well-characterized visual system of vertebrates. A comparative neurobiological approach may therefore be able to separate universal principles underlying visual processing from group-specific features. Classical anatomical work on this region has recently been supplemented by genetic, transcriptomic, and functional investigations of this brain region. To date, however, we lack a functional description at cellular resolution. We developed a method to head-fix awake juvenile squids (S. lessoniana) and perform extracellular electrophysiological recordings from the OL using Neuropixels probes. Using various visual stimulation experiments, we have identified the neuronal tuning and receptive field properties of neurons across the different layers of the OL. We have anatomically mapped the location of hundreds of neurons, using tissue clearing and light-sheet microscopy. Our functional atlas reveals a hierarchy of visual information processing within the OL. Together, our study presents a significant methodological and biological advance in understanding the cephalopod visual system.

Poster Session 1 | Poster Wall 224 | Label: PS1.224

Category: Vision and photoreception

Influence of temperature on motion processing in the central brain of bumblebees

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Honeybees and bumblebees can regulate their body temperature independent of ambient temperature (Heinrich, 1974, Science). Nevertheless, there is only a limited number of studies investigating the effects of temperature on sensory processes and neuronal coding. In bumblebees, photoreceptor responses are faster at higher temperatures independent of whether temperature is actively increased during walking or passively due to an external heat source (Rother and Müller et al., 2023, Proc. R. Soc. B). Likewise, in blowflies, photoreceptors (Tatler et al., 2000, J. Comp. Physiol. A) and visual interneurons (Warzecha et al., 1999, J. Exp. Biol.) show faster responses at higher temperatures. Here we investigated the effect of temperature on the tuning of wide-field motion-sensitive neurons in the central brain of bumblebees. Using tetrodes we examined responses to gratings with different temporal and spatial frequencies under different temperature conditions (24°C and 32°C). For most of the units, we observed three effects. First, the ongoing activity was increased at the high temperature condition. Second, the spike rate during stimulation was increased (also after baseline-correction). Third, the preferred velocity of the units was shifted to higher values at the high temperature condition. It is likely, that the neuronal activity we measured stemmed from neurons of the optic lobes that are involved in the control of self-motion. Both walking and flying increases the head temperature of bumblebees and at the same time leads to higher rates of visual input. Therefore, both the increased sensitivity and the shift of the sensitivity maximum to higher temperatures might be beneficial during locomotion and suit the requirements during these behaviours.

Poster Session 1 | Poster Wall 225 | Label: PS1.225

Category: Vision and photoreception

Lamina neurons build the basis for dynamic processing in the hawkmoth visual system

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Many animals strongly rely on their visual sense, as it provides information about the natural environment with particularly high dimensionality. Natural visual scenes contain different spatial frequencies, which can vary across the visual field (Bigge et al., 2021, Curr Biol 31, 6: R280-R281), while changing light conditions affect the reliability of the visual signal. This complex sensory input requires a high amount of flexibility in the visual system.

Hawkmoths provide a prime example for dynamic visual processing: the motion neurons in the lobula complex are sensitive to contrasts over a million-fold range of light intensities (Stöckl et al., 2017, Proc R Soc B 284: 20170880). These properties can only be explained by early dynamic processing of the visual input. An important neuropil for such is the lamina, the first processing stage in the insect visual system. Its main relay neurons, the lamina monopolar cells (LMCs), receive information directly from the photoreceptors and play an important role in shaping contrast, luminance, spatial and temporal information.

To investigate the role of LMCs in visual processing, we used serial block-face scanning electron microscopy to reconstruct the anatomical fine structure of the lamina in a nocturnal and diurnal hawkmoth species. These allow us to identify pre- and post- synaptic sites among the LMCs and photoreceptors. Based on these connectomics, and the morphology of the different neuron types, we establish a new lamina neuron classification for hawkmoths. Further, we discuss striking differences in LMC anatomy and connectivity within homologous neurons of closely related species and set these into context with their visual processing functions.

Poster Session 1 | Poster Wall 226 | Label: PS1.226

Category: Vision and photoreception

Hoepel 1 is a potential Rhodopsin interacting protein required for maintenance of Drosophila photoreceptor cells

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Rhodopsin (Rh1) is a transmembrane protein that functions as G protein-coupled receptor and constitutes the major photopigment in Drosophila melanogaster compound eye. As a photoreceptor, it interacts with a G protein to activate visual signal transduction and with Arrestins to terminate activation. Additionally, Rh1 associates with numerous other proteins during its turnover, including the process of folding in the endoplasmic reticulum, vesicular transport, receptor internalization from the plasma membrane and degradation. To identify potential in vivo Rh1 interaction partners in these biological processes, we expressed a Rh1-TurboID fusion protein (Rh1::TbID) construct in Drosophila photoreceptors that enabled proximity labeling of prospective targets (Feizy et al., 2024, Scientific Reports 14:1986). In this screen we identified Hoepel 1 (hoe1), a previously uncharacterized protein and an orthologue to human OCA2 (OCA2 melanosomal transmembrane protein), as a Rh1 interaction partner. To investigate Hoe1 connection to Rh1, hoe1 was “knocked-out” in the Drosophila eye using a tissue specific CRISPR-Cas9 system and the resulting mutants were analyzed for changes in Rh1 levels, phototransduction performance and retinal health.

Western Blot analysis revealed that overall Rh1 amounts decrease with increasing age of the fly. Electroretinograms of mutants of various ages also showed age related lessening of phototransduction performance, suggesting a possible degeneration of the rhabdomeres. This possibility was investigated further with water immersion microscopy followed and histochemistry. Both approaches revealed a significant increase of age-related photoreceptor degeneration in hoe1 mutants, when compared to wildtype flies of the same age.

Poster Session 1 | Poster Wall 227 | Label: PS1.227

Category: Vision and photoreception

Ancestral cones differentially drive and regulate retinal motion vision circuits

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Most extant animal eyes leverage at least three main axes of variance in natural light: space, time, and color. Of these, diverse combinations of space and time centrally underpin complex visual behaviors, while spectral information is rarely thought to be central in such circuits. Additionally, both from basic physics of how light travels in the water where vision first evolved, and from insights into the evolution of vision itself, spectral and spatiotemporal information are fundamentally entwined. Beyond color vision, the purpose of spectrally distinct cone-types may be to serve as distinct feature channels that differentially underpin diverse non-spectral aspects of vision. Here, we test the hypothesis that computations in widefield- and object-motion mainly draw on ancestral red (LWS) and UV (SWS1) photoreceptors, respectively, while green (RH2) and blue (SWS2) cones are primarily used to regulate rather than generate visual responses.

We use zebrafish transgenic lines for acute cone-type ablations combined with physiological and behavioral recordings. We perform 2P imaging across the live zebrafish brain during spatially and spectrally patterned visual stimulation, in the presence and absence of specific cone-types. We then probe how different behaviors, such as the optomotor reflex, phototaxis and water column swimming behavior, are affected by the absence of different cones.

These new insights, strongly support the idea that in zebrafish, LWS- and SWS1-driven circuits centrally underpin widefield- and object-motion encoding, respectively, while RH2- and SWS2-circuits primarily act in a suppressive manner to regulate LWS- and/or SWS1-circuits.

Poster Session 1 | Poster Wall 228 | Label: PS1.228

Category: Vision and photoreception

Optic flow neurons in the pretectum have different direction tuning at very fast speeds

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The pretectum contains neurons responsive to global visual motion. These signals are sent to the cerebellum, forming a subcortical pathway for processing optic flow. Global motion neurons exhibit selectivity to both direction and speed, but this is usually assessed by first determining direction preference at intermediate velocity (16-32 deg/sec), and then assessing speed tuning at the preferred direction. A consequence of this approach is that it is unknown if direction preference changes with speed. We measured direction preferences in 114 cells from 44 zebra finches Taeniopygia guttata across a range of spatial and temporal frequencies. The cells showed highest overall activity at intermediate speeds (32 deg/s) with lower overall activity as speed increased or decreased. 15% of the cells were omnidirectionally excited across most speeds. The remaining 85% of the cells had direction tuning that changed with speed. For at least at one tested speed, some cells were directionally-selective, some were bi-directional-selective, and some were omnidirectionally excited. One third of the cells were either directionally- or bidirectionally-selective at intermediate speeds and became omnidirectionally excited at fast speed (1024 deg/sec). Collectively, these results indicate that pretectal global visual motion neurons are most responsive at the stimulus speeds typical for locomotion, but that a large fraction of the cells also respond omnidirectionally, especially to fast speeds that could signify impending collisions.

Poster Session 1 | Poster Wall 229 | Label: PS1.229

Category: Vision and photoreception

Unravelling the transformation of skylight polarization signals into a neural compass in the bumblebee brain

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Bumblebees use a variety of celestial cues, including the polarization pattern of the sky, to guide their flight direction during navigation. Anatomically, the polarized light (POL) input pathway is well described in the bumblebee brain. Yet, since the bumblebee polarization vision pathway lacks functional characterization, we set out to unravel how the brain of this expert navigator uses POL to generate a robust internal compass. We designed a novel rotating skylight stimulus, allowing us to present a wide range of non-axially symmetrical polarization patterns in the UV range. These are complemented with optic flow stimuli based on dead leaves patterns, mimicking naturalistic image statistics, presented on a 360˚ LED display. Both stimuli combined enable us to characterize the parallel visual input pathways connecting the early visual centres with the central complex (CX), a highly conserved brain region controlling navigation. A current computational model of the POL input pathway (Gkanias et al, 2019) provides a solution for how a view of the polarization pattern of the sky can be transformed into a 360˚ compass representation of azimuthal space. We use intracellular electrophysiological recordings to attribute a function to each step of the POL input pathway and thereby biologically constrain the above mentioned computational model. At the same time, the model is used to predict neural activity for specific visual input stimuli, thus creating a feedback loop between experimental and computational approaches to bumblebee vision. Understanding which information is encoded in the parallel pathways towards the CX and how these compass signals relate to each other will provide insight on how bumblebees generate a robust heading representation in variable environments. The resulting biologically constrained model of POL processing will directly contribute to the development of highly efficient nanophotonic navigation circuits inspired by insect neuroscience.

Poster Session 1 | Poster Wall 230 | Label: PS1.230

Category: Vision and photoreception

Visual physiology of two sympatric wasps: Survival-adapted vision

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An organism's sensory structures and physiology are tuned to its ecology. Most animals rely on vision to perform various behavioural tasks, including navigating, foraging, mating, and communicating. Variations in the anatomy and physiology of the eye likely reflect differences in the habitat and life history of the species. We studied two sympatric species of wasp, which differ in prey preference, nesting behaviour, and ecological roles: The social and euriphagous Polistes humilis (Vespidae) and the highly specialised araneophagous Sceliphron formosum (Sphecidae). We described and compared the structure of their compound eyes, the number of ommatidia and lens diameter. Here, we first used pattern electroretinography (pERG) to measure the wasps' compound eyes' spatial resolving power and contrast sensitivity. The wasp species' visual physiology differed, with the solitary hunter S. formosum having a more acute vision. This comparative study showcases the different visual physiology that sympatric wasps have evolved that match their unique visual ecologies.

Poster Session 1 | Poster Wall 231 | Label: PS1.231

Category: Vision and photoreception

A competitive disinhibitory network for robust optic flow processing in Drosophila

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Sensory experience critically depends on our movement: the brain must incorporate information about self-movement to guide behavior, or discount self-generated sensory signals to detect external events in the world. How do sensory circuits process self-generated signals in different ways to meet specific demands?

Lobula Plate Tangential Cells (LPTCs) of the fly optic system respond to visual motion generated by self-movement, a.k.a. optic flow. LPTCs also receive extra-retinal signals related to head/body movements during locomotion, which suppress or enhance their activity depending on the task. This makes LPTCs ideal for studying task-dependent processing of self-generated signals. Although some pathways that connect LPTCs to head rotations have been identified, the full circuitry enabling optic flow signals to control behavior remains largely unidentified.

Using electron microscopy datasets of the adult Drosophila brain, we identified synaptic partners of canonical LPTCs, Horizontal (HS) and Vertical System (VS) cells. We found that HS and VS cells are connected recurrently to a set of GABAergic interneurons at their axons. These interneurons are sensitive to specific optic flow patterns that are either induced by forward or turning movements, revealed by functional imaging. Each class is different from each other and from LPTCs in their optic flow sensitivity, which is at least partially generated by their recurrent inhibitory interactions.

The circuit architecture and functional responses together suggest that these LPTC circuits can be used to monitor the angular deviations and elicits turns to maintain a straight course. Altogether, our results reveal central networks that process complex patterns of optic flow field into translational and rotational directions, which in combination with extra-retinal signals, modulate the activity of visual neurons according to the ongoing task of an animal moving through space.

Poster Session 1 | Poster Wall 232 | Label: PS1.232

Category: Vision and photoreception

Pursuit characteristics and sensory feedback shape evasive strategies in larval zebrafish

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Animals must react to threatening stimuli in a way that increases their chance of survival. The visual loom is a known threatening stimulus that emulates an approaching predator and mainly elicits strong escape responses away from the stimulus in larval zebrafish. However, the behavioral space of the response to the visual looms may also involve freezing and other types of swim bouts. In this work, we carried out free swimming behavioral experiments and immunofluorescence imaging protocols to explore the differences between types of visual looms and how they affect behavioral output through habituation and changes in internal states. We compared three types of visual looms: avoidable, inescapable, and escapable. In our habituation stimulus train, we observed differences in the proportion of escape responses and freezing behaviour between these paradigms and notable differences in the extent of habituation after 3 blocks of 10 visual loom presentations. By classifying the observed swim bout types and observing the swim bout sequences, we also found differences in bout transition probabilities between paradigms that may indicate that each paradigm causes different shifts in internal states: inescapable looms cause greater habituation, whereas escapable looms increase arousal and avoidable looms could contribute to anxiety like behaviour and helplessness. Our pERK/tERK immunofluorescence analysis indicates that the habenula and thalamus could be involved in these shifts in behaviour. In future work, we aim to evaluate the same paradigms using whole-brain two-photon calcium imaging and virtual reality stimulus controls to further elucidate the neural circuits involved in habituation and shifts in internal states.

Poster Session 1 | Poster Wall 233 | Label: PS1.233

Category: Vision and photoreception

Neural substrates of visuo-behavioural changes during frog metamorphosis

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Animals interpret incoming sensory information in the context of their own, ever changing visual ecology. For example, where tadpoles may see each other as conspecifics, a frog may see food. What are the visual circuit differences underlying such alternative interpretations of the same object? Working on the frog Xenopus laevis, we study the neural underpinnings of changes in visual behaviours as the same animal transitions from a shy filter feeder to a ferocious ambush predator during metamorphosis. To this end, we begin by characterising the time course of behavioural changes from postembryonic larvae to juvenile frogs.

We use close-loop video tracking of freely swimming animals to characterize reactions to visual threats and prey-like stimuli, in presence or absence of a 'visual shelter'. While looming stimuli reliably induces escapes in young tadpoles 2-4 weeks post fertilization (WPF), escape probability is reduced in pre-metamorphic tadpoles (5-6 WPF) and near absent in froglets (12+ WPF). In isolation, prey-like stimuli induce no reaction in tadpoles nor in young froglets. However, this dramatically changes upon the addition of a 'visual shelter' (a large static dot), whereupon frogs reliably perform prey-capture behaviour from late metamorphic stages. These results suggest that escape behaviours are strongest in young larvae, in line with their free-swimming lifestyle, and then gradually decline. Vice versa, prey capture rapidly emerges after the metamorphic climax, but requires the presence of shelter in line with a stationary lifestyle.

As a next step, we have established ex-vivo eye-and-brain preparations, custom 2-photon imaging methods and a new transgenic Xenopus line that allow functional imaging of visual circuits across development, to identify the neural correlates that facilitate behavioural reprogramming.

Poster Session 1 | Poster Wall 234 | Label: PS1.234

Category: Vision and photoreception

Camouflaging motion in the hunting display of broadclub cuttlefish

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Movement often gives away the presence of cryptic predators to their prey. Some predators are known to use background motion to hide their movement (Collett and Land 1978, Srinivasan and Davey 1995, Mizutani, Chahl et al. 2003, Kane and Zamani 2014, Bian, Elgar et al. 2016). It has also been proposed that some predators may use coloration and behaviour to impair detection or recognition of their movement by generating their own motion noise, for example by using protean movement strategies (Richardson, Dickinson et al. 2018), generating motion confusion with high contrast markings (How and Zanker 2014, Hall, Cuthill et al. 2016, Merilaita, Scott-Samuel et al. 2017, Scott-Samuel, Caro et al. 2023), dynamically changing colour (Duarte, Flores et al. 2017, Kjernsmo, Hall et al. 2018, Cuthill, Matchette et al. 2019) or using movement-based flicker-fusion camouflage (Umeton, Tarawneh et al. 2019). Here, we provide evidence for the use of dynamic skin patterns by broadclub cuttlefish to camouflage their hunting manoeuvres. When stalking crabs, the broadclub cuttlefish passes contrasting dark stripes in a downward direction across the head and arms until it strikes its prey (How, Norman et al. 2017). First, we show that crabs are less likely to respond to approaching predator stimuli when they feature downwards moving stripes. Second, we reconstruct the approach trajectories of hunting cuttlefish in the wild and show that they use moving stripes only when closer to the target, and that the temporal frequency of the stripes is influenced by the approach speed of the cuttlefish. Third, we show that the stripe display produces a motion pattern that is different to that of looming predators. In conclusion, we present independent, yet interconnected evidence that this dynamic stripe display camouflages the cuttlefish’s motion cues during the last moments of approach, most likely by overwhelming the looming motion signal with strong and non-threatening downward motion noise.

Poster Session 1 | Poster Wall 235 | Label: PS1.235

Category: Vision and photoreception

Animacy perception in the common cuttlefish, Sepia officinalis

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Recent studies have shown that cuttlefish, even without prior learning, can visually differentiate between prey and predators and adjust their behaviours accordingly. However, the precise visual cues and neural mechanisms underlying this capacity remain poorly understood. Research in terrestrial vertebrates, from primates to birds, has revealed that animacy cues – specific shapes or motion types typical of living organisms – serve to identify living organisms in nature. Whether animacy cues are also used by cuttlefish, a species from a distinct phylum, is an open question. This study explores whether newly hatched and visually inexperienced cuttlefish utilize motion types akin to those observed in terrestrial vertebrates for the detection of living entities in their surroundings (prey or predators). Additionally, it seeks to elucidate the neural mechanisms underlying animacy perception. Using video screens, we exposed cuttlefish to stimuli mimicking the movements of typical prey and predators and measured their behavioural responses. Thereafter, we compared the number and localisation of activated neurons using immediate early gene product labelling. The insights gained from this research will advance our understanding of animacy perception evolution and its underlying mechanisms.

Poster Session 1 | Poster Wall 236 | Label: PS1.236

Category: Vision and photoreception

The impact of brain injury and regeneration on visually guided behaviors in the axolotl (A. mexicanum)

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Despite the axolotl's exceptional ability to regenerate complex tissues, little research has focused on this phenomenon in the brain. While regeneration seems to restore the overall tissue morphology and original diversity of cell types in the brain, studies have revealed that the organization of cells within the tissue is not re-established in the same way. We aim to understand whether the morphological repair observed during axolotl brain regeneration is accompanied by a functional restoration of neuronal circuits and ultimately the behaviors associated with it.

Recently, we have established a system to investigate visually guided behaviors in the axolotl where we can give the animals a visual stimulus projected onto their tank and record their response. With this, we successfully performed first evaluations of the animal's preferred prey characteristics. In a next step, we aim to expand our assay to other behaviors such as predator avoidance, phototaxis or an optomotor response.

We will combine the behavior assay with other tools such as in vivo 2-photon calcium imaging to visualize neuronal activity and optogenetic stimulation to map the axolotl's visuomotor circuit.

The circuit will then be perturbed by injuring the optic tectum, the area where most of the visual processing is thought to occur, and followed throughout regeneration. Using the tools mentioned above, the extent of functional restoration of the visuomotor circuit will be evaluated both on the level of neuronal activity, as well as the behavioral output.

Poster Session 1 | Poster Wall 237 | Label: PS1.237

Category: Vision and photoreception

“Retina-like” functional and cellular complexity in the zebrafish pineal gland

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The vertebrate pineal gland (PG) is a neuroendocrine structure that mediates the effects of the circadian clock through melatonin secretion. In many non-mammalian vertebrates, the PG is located dorsally, where it is directly exposed and sensitive to environmental light. However, much of the sensory function of PG neural circuitry remains to be explored. We therefore ask: What are the functional signatures of light driven responses in PG neurons, and to what extent do they mimic those of the retina? Further insights promise a renewed understanding of the possible evolutionary history of the vertebrate lateral eye. We present our early insights into the functional organisation of the zebrafish PG, and a first take anatomical characterisation.

We use two-photon imaging in larval zebrafish to measure light-evoked calcium responses of distinct populations of genetically targeted PG neurons in vivo, including rod- and cone-like photoreceptors (PRs) and putative projection neurons.

First, we find most “cone” PRs negatively encode luminance information (OFF responses) with surprising vigour and speed, responding to temporal changes in luminance up to at least 5 Hz. Moreover, “cones” encode both positive and negative changes in luminance contrast in a graded, nonlinear manner. In addition, we identify a small proportion of ON responses, possibly accounting for PRs expressing parietopsin and parapinopsin.

Second, we detect OFF responses in “rod” PRs which appear to be several-fold more light-sensitive compared to “cones”.

Third, we find ON and OFF calcium responses in various PG projection neurons, perhaps hinting at surprisingly complex and diverse local processing.

ogether, we find several indications that contrary to the prevailing view, PG circuitry is retina-like in function. We are following up with detailed anatomical reconstructions, an expansion of the visual coding space, and pharmacological manipulation targeting putative retinal neurotransmitter systems.

Poster Session 1 | Poster Wall 238 | Label: PS1.238

Category: Vision and photoreception

Motion Salience and the Evolution of Alerting Displays in Habronattus Jumping Spiders

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Signals must effectively propagate through the environment to reach intended receivers. In cluttered and/or noisy environments, signaling bouts often begin with alerting display elements that capture initial receiver attention. Such alerting displays are posited to be under strong selection to 'stand-out' against environmental noise, such as habitat background motion. This suggests that species differences in alerting displays might evolve in response to differences in environmental noise. We investigated this possibility in Habronattus jumping spiders. Male Habronattus courtship often begins with large foreleg waves which serve to alert prospective mates to their presence. Previous research in H. pyrrithrix has found that male displays that include alerting waves are more successful in drawing female attention. Further, Habronattus species differ in both the motion characteristics of their alerting display waves and the background motion properties typically found in their habitats. We therefore hypothesized that diversity in the alerting displays of Habronattus species may have evolved in response to variation in the background motion typically found in their habitats. Here, we first quantified key motion characteristics of alerting displays in 12 species of Habronattus spiders. We then quantified motion characteristics of their habitats. Combining these datasets with an established phylogeny, we investigated whether the characteristics of alerting displays have evolved to 'stand-out' against background habitat motion in this group. Our results provide critical insight into the role of habitat motion in the evolution of motion-based display diversity, offering a new lens on the evolutionary dynamics of complex visual displays.

Poster Session 1 | Poster Wall 239 | Label: PS1.239

Category: Vision and photoreception

Amphibious compound eyes: shifts in eye morphology and function in Belostoma flumineum

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Belostomatids (Hemiptera: Belostomatidae), commonly known as giant water bugs, are insects that are fully aquatic as nymphs and amphibious as adults. At all life stages, belostomatids use vision to hunt in their aquatic environments. As adults, belostomatids can leave the water to navigate the terrestrial environment in search of resources. To test if belostomatid visual systems are adapted to these variable environments, we examined eye morphology, optical performance, and visually-guided behaviors in Belostoma flumineum throughout ontogeny by studying first and third (of five) nymphal instars and adults. We measured radius of curvature of the external corneal surface and found that first instars had round corneas (7.04 + 1.52 μm), third instars had less round corneas (31.55 + 29.58 μm), and adults had flat corneas (172.42 + 78.14 μm). In amphibious animals, flat corneas allow eyes to have similar refractive power in both the aquatic and terrestrial environments they inhabit. Next, we used a modified hanging drop method to measure focal lengths in nymphs and adults. We found that the corneas of nymphal instars focus light better in water (first instar: 53.65 + 22.12 μm; third instar: 53.22 + 13.57 μm) than in air (first instar: 32.89 + 10.1 μm; third instar: 43.57 + 4.15 μm). In contrast, adult corneas focus light equally well in air and in water (44.03 + 5.34 μm and 40.88 + 2.18 μm, respectively). To ask if these adaptations affect visual performance, we used an optomotor assay to assess the visually-guided behaviors of B. flumineum in air and water. We found that nymphs follow a visual stimulus in water (p B. flumineum are adapted to function optimally in water while the eyes of adults are modified to support vision in both aquatic and terrestrial environments.

Poster Session 1 | Poster Wall 240 | Label: PS1.240

Category: Vision and photoreception

Visual responses and adaptation in looming sensitive descending neurons

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Responding to looming motion is vital to the survival of many animals, as this may represent an approaching predator. In insects, there are thus many looming sensitive neurons. Looming-sensitive descending neurons (DNs) connect the sensory centers in the brain with thoracic ganglion motor regions. Looming-sensitive DNs are classified by a stronger and faster response to a looming stimulus compared with a motion-free control [1].

Here, we record from looming-sensitive DNs in female hoverflies (Eristalis tenax), which are highly visual and amenable to electrophysiological and behavioral investigations. Using intracellular electrophysiology and dye filling, the neurons are characterized anatomically and physiologically. Upon classification as a looming-sensitive neuron, we present two additional stimuli: small, black targets (3°x3°) in 4 different directions and full-screen sinusoidal gratings (0.1 cpd, 5 Hz) in 8 directions. Out of 50 recorded looming-sensitive DNs, 50% additionally respond to small targets and gratings, 30% to small targets but not to gratings, with the remaining 20% looming selective. Morphological reconstructions also highlight the diversity, with large variation in location and branching of both input dendrites and axon terminals.

While the morphological and physiological diversity highlights the range of behaviors these neurons need to support, they all adapt to repetitive stimuli (expansion of black circle on a white screen: 1° to 117° over 1 s, $l/|v|$ = 10 ms; then remaining stationary for 1 s, followed by a white screen for 1, 3, 10 or 20 s, repeated at least 7 times). Indeed, all looming-sensitive DNs show a strong response decrease across repetitions when the break is less than 3 s. With break times of 20 s, there is no adaptation. Using open loop behavioral experiments, we are currently investigating whether these findings are matched by behavior.

[1] Nicholas et al, J Comp Physiol A, 2020.

Poster Session 1 | Poster Wall 241 | Label: PS1.241

Category: Vision and photoreception

Polarisation and luminance contrast are processed in the same way by escaping fiddler crabs

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Many invertebrates such as crabs and insects have two-channel polarisation systems that make their vision sensitive to polarisation information across their full visual field. It is increasingly clear that object-based polarisation vision plays a significant role in the behavioural decisions made by these animals. Yet, whether that polarisation contrast is analysed independently from luminance contrast, or whether both forms of contrast contribute to a single channel is still unclear. Fiddler crabs have been shown to escape both from pure luminance as well as pure polarisation stimuli and there is evidence that they process both forms of contrast in parallel rather than combining them in a single channel. Given the different nature of the two forms of contrast, their differing reliability and distribution across the animals' environment, we wanted to test whether the crabs use the same decision criterion when timing their escape run regardless of contrast source. We have recently shown that fiddler crabs time their escape run from a directly approaching luminance stimulus by the speed with which the stimulus expands on their retina. A decision criterion that makes them unique amongst invertebrates. We show here that these crabs use the same decision criterion when faced with a pure polarisation stimulus, suggesting that the escape decision is processing polarisation and luminance information in the same way. However, the results also suggest that polarisation contrast is much less effective in triggering escape responses than luminance contrast. Together these results suggest that the two sources of information must initially be separated into two distinct pathways to derive independent measures of contrast, but then are likely re-combined at or before entering the lobula giant neurons that control the escape response.

Poster Session 1 | Poster Wall 242 | Label: PS1.242

Category: Vision and photoreception

Species specific light reactions of Arctic Calanus copepods revealed by AI assisted tracking

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Current environmental changes are affecting the light climate in the Arctic Ocean and likely challenging the organisms exhibiting light guided behaviors. Calanoid copepods are the main component of zooplankton biomass in the Arctic Ocean and are suggested to follow a certain light isocline, but little is known about the temporal aspects of their light reactions and differences between species. In this study, behavioral experiments to determine the visual sensitivity and light reactions of the species pair Calanus glacialis/finmarchicus collected from Arctic fjords were conducted at three different time points: polar night, polar day and close to the autumnal equinox. In the experiments a batch of animals was exposed to an increasing intensity series of 10 second white light stimuli presented every 2 minutes and their reactions filmed in infrared light. The movement of each individual was tracked by SLEAP, an open-source deep-learning-based framework for multi-animal pose tracking, before, during and after each stimulus. The results showed that both baseline swimming activity and light reactions differed between the species, the more Arctic C. glacialis being more active and showing clearer photoreactions throughout the year. Interestingly, in some experiments the animals were more strongly activated by light increments and in others by light decrements, indicating that different signaling pathways are activated depending on season or physiological state.

Poster Session 1 | Poster Wall 243 | Label: PS1.243

Category: Vision and photoreception

Glowing and seeing red: molecular evolution of bioluminescence in dragon fishes

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In the ocean depths, particularly within the mesopelagic zone ranging from 200 to 1000 meters, light is scarce, and limited to the blue-green spectrum. In this dimly lit environment, most species capable of emitting light (bioluminescence) predominantly emit in the blue spectrum. Some species of dragon fishes (Stomiiformes), one of the most diverse deep-sea fish order, stand out by their exceptional ability to emit and see bioluminescence in the (far) red spectrum. The luciferase enzyme emits in the short (=blue) wavelengths, which are then converted into the longer (=red) wavelength by another enzyme binding phycocyanobilin as a chromophore phenomenon called Bioluminescence Resonance Energy Transfer (BRET). Additionally, the spectrum is further filtered by the cut-off filter in one species (Malacosteus niger). We employed a combination of transcriptomics and proteomics on samples of deep-sea fish (Stomiiformes) that were sampled in the Atlantic. We specifically focus on the blue and red photophore and the surrounding tissue and we aim to identify the candidate gene(s) associated with the luciferase enzyme and/or its pathway by the differential gene expression analysis. On top of this, we aim to search for a set of genes specific for the (far-) red glowing species compared to the "blue-only" species (Argyropelecus, Photostomias) to identify the putative candidate for converter enzyme, to test if the luciferase is the same for both blue and red photophores and to provide a comparative framework. Overall we aim to elucidate the molecular mechanisms underlying bioluminescence in this fascinating group of fish.

Poster Session 1 | Poster Wall 244 | Label: PS1.244

Category: Vision and photoreception

I see you in green: The story behind RH2 opsin gene duplications in European cypriniform fishes from family Leuciscidae

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Vision plays a key role in life of many vertebrates, fish included. A photoreceptor cell layer in retina allows for colour perception mediated by cones, and scotopic vision in dim-light conditions mediated by rods. In the cones, four types of the opsin genes are expressed (SWS1, SWS2, RH2 and LWS), while one type is expressed on rods (RH1). Some cypriniform fishes acquired multiple copies of these genes during evolution (predominantly RH1 and RH2 opsins), and they thus express alternative opsin combinations specific to different developmental stages of the fish. In this study, we focused on Central European cypriniform fishes predominantly from family Leuciscidae (14 species out of 22 studied), and we report the plasticity of the visual system comparing data of adults and juveniles of 11 species. We sequenced the retina transcriptomes, whole genomes, and we applied the FISH on the photoreceptors. In adults, the most abundant opsin in retina is the long wavelength sensitive LWS opsin, which is consistent with the freshwater turbid environment in European rivers. The LWS opsin gene is expressed in double cones of the retina while the other cone from the pair carries RH2 opsin. Larvae and juveniles, however, predominantly express shorter wavelength-sensitive opsins (SWS1, SWS2) corresponding to the conserved cone development in vertebrates, and probably adaptive as they linger close to the surface on shallow riverbanks. These short wavelength-sensitive opsin genes are expressed on single cones. Interestingly, the most variability among species was found in numbers of green-sensitive RH2 opsin genes, when even closely related species differ substantially in their expression profiles. Our combined genomic and transcriptomic data suggest a presence of five ancestral RH2 gene copies in family Leuciscidae, some of which have further diversified in the extant species. In other cypriniform fishes, we detected both ancestral and recent duplications of the green opsin gene.

Poster Session 1 | Poster Wall 245 | Label: PS1.245

Category: Vision and photoreception

Shark Vision – What does the retina tell the brain?

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The basic blueprint of retinal organisation is highly conserved across all vertebrates. And yet, our detailed understanding of its functional properties have primarily come from only a handful of species that can not reflect the diversity of neither the lifestyle and behavioural repertoire of vertebrates nor the environments they inhabit.

In search of ancestral in origin, universal principles of vertebrate visual processing, we explore the surprisingly understudied elasmobranch tree, the first branch of jawed vertebrates diverging 440 million years ago, with species known to possess elaborate optics but historically assumed of having poor vision.

We characterise the retinal output of the catshark Scyliorhinus canicula by presenting a broad arsenal of visual stimuli and recording with multi-electrode arrays simultaneously from hundreds of retinal ganglion cells. We demonstrate that a sophisticated and well-studied computation, namely direction selectivity, is an ancient feature of the vertebrate retina and confirm, in line with the general concept, the early segregation of visual information in opposite elementary channels: on and off, transient and sustained. Upon pharmacological manipulations however, and contrary to the other vertebrates, we unveil unexpected crosstalk between the different channels in the shark retina.

Poster Session 1 | Poster Wall 246 | Label: PS1.246

Category: Vision and photoreception

Diversification of outer retinal feature channels through bipolar cells in larval zebrafish

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A new theory of vertebrate vision (1) suggests that 'beyond colour', cone types ancestrally serve as parallel feature channels at the retinal input. Bipolar cells (BCs) then distribute the combined and contrasted cone signals across the layers of the inner retina, in effect routing different cone circuits onto specific behavioural programmes. Here, we study this process in the retina of zebrafish, an aquatic species that retains the full ancestral photoreceptor complement and which thrives in a visual world comparable to when vision first evolved. We systematically chart spatial, temporal and chromatic receptive fields of BC terminals in larval zebrafish using in vivo 2-photon calcium imaging – a first in an ancestrally tetrachromatic vertebrate.

We find a high degree of functional heterogeneity across the population, following systematic functional and anatomical rules. For example, receptive field sizes and temporal properties were heterogeneous at long wavelengths but homogeneous at short wavelengths, supporting the idea that 'general purpose' vision is mainly driven by the ancestral red system while the UV-system serves more specialised functions. Moreover, many BCs are centre-opponent, and this property is largely impervious to pharmacological blockage of amacrine cells. This strongly suggests that spectral interactions at the level of BCs are inherited from the outer retina. Moreover, occasional surround structures tended to be surprisingly asymmetrical, contrary to the prevailing expectation but in line with recent work on mouse ganglion cells (2). In sum, our evidence suggests the parallelisation of feature channels starting from photoreceptors is consolidated and diversified within the receptive fields of bipolar cells.

1. Baden, T. Ancestral photoreceptor diversity as the basis of visual behaviour. Nat. Ecol. Evol. 1–13 (2024)

2. Gupta, D. et al. Panoramic visual statistics shape retina-wide organization of receptive fields. Nat. Neurosci. 26, 606–614 (2023)

Poster Session 1 | Poster Wall 247 | Label: PS1.247

Category: Vision and photoreception

Parallel pathways for visual and olfactory information in the mushroom bodies of the swallowtail butterfly brain

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The Japanese yellow swallowtail butterfly (Papilio xuthus) is a flower-foraging insect with sophisticated color vision. In female butterflies, innate color preference is modified by certain plant odors, suggesting integration of visual and olfactory information in the brain. Likely candidates for olfactory-visual signal integration are the mushroom bodies (MBs), prominent paired neuropils of the insect central brain. In P. xuthus, both visual and olfactory afferents supply the MB input site, the calyx, but terminate in different concentric calycal zones, spatially segregated from each other. The organization of the MB output site, the lobes, are poorly understood in the butterfly, but seem to exhibit further structural specifications, quite different from the bifurcated lobes of other insect MBs. Are the modality-segregated inputs integrated within the MBs, and where are the projection sites of MB output?

Here, we investigated the detailed architecture of the butterfly MB. First, we performed a three-dimensional reconstruction of the MB and other brain neuropils based on confocal slices of a wholemount preparation immunolabeled by anti-synapsin and anti-FMRFamide. Next, we traced the axon-like processes of Kenyon cells, the MB intrinsic neurons, by iontophoretic injection of neurobiotin to the MB calyx, which labeled a small number of Kenyon cells per preparation. The results suggest a parallel arrangement of Kenyon cells and a compartmental organization of the lobes, but modality segregation in the lobes is less clear than in the calyx. Further, by injecting the tracer into the MB lobes, we found at least two MB output pathways connecting the lobes to other sensory integration sites of the central brain: the anterior optic tubercle and the lateral horn. Our findings facilitate further anatomical studies as well as future physiological and functional investigations of multisensory integration in the butterfly brain.

Poster Session 1 | Poster Wall 248 | Label: PS1.248

Category: Vision and photoreception

Insect retinal movements in the context of visual ecology and evolution

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Many animals are capable of eye movements with a multitude of functions being hypothesized and tested. Insects, however, have been traditionally viewed as being incapable of moving their eyes independently of the head. This assumption stems from the rigid anatomy of compound eyes but is still surprising considering the unrivaled insect biodiversity and concomitant variations in visual ecology.

Indeed, one group of insects – true flies (Diptera) – has been shown to possess retinal muscles capable of shifting the photoreceptor tips. This shift of the photoreceptor axis in Diptera has similar consequences to eye movements in lens eyes. Retinal movements have been functionally characterized recently in the fruit fly Drosophila melanogaster, and remarkably, these flies exhibit active visual strategies that are reminiscent of vertebrate eye movements. It remains to be discovered, whether retinal movements are unique to Diptera or even only true flies from the infraorder Muscomorpha.

The primary goal of my project is to explore the variation (if any) of retinal movements across insect orders and Diptera species. Species sampling across taxa with decreasing evolutionary distance will allow to differentiate variations of retinal movements attributable to evolutionary and ecological factors. This will bring us closer to ultimately understanding the function and role of retinal movements in insect vision.

So far, I have obtained behavioral evidence for retinal movements and/or anatomical evidence for retinal muscles across 24 out of 29 insect orders as well as most of Diptera infraorders.

This preliminary data suggests, perhaps surprisingly, that retinal muscles have evolved late, as putative retinal muscles were only found in the sister group of Diptera – scorpionflies (Mecoptera). In contrast, Diptera presents remarkable variation of retinal muscle presence, number and size, as well as sexually dimorphic retinal muscles in some species.

Poster Session 1 | Poster Wall 249 | Label: PS1.249

Category: Vision and photoreception

Investigating the Role of Double Cones in the Frog Retina

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Double cones are perhaps the single most important "evolutionary invention" of the tetrapod eye. They are absent in fish, but present in amphibians, reptiles, birds, and non-eutherian mammals, implying that they evolved soon upon the first emergence of vertebrates on land. In many species they are numerically dominant, and yet, we still do not truly understand what exactly they do, or how. Here we take a renewed look at this long-standing question, leveraging the experimental accessibility of xenopus as a model. To this end, we employ connectomic level circuit mapping with multielectrode array recordings from the retinal output during spectrally and spatially patterns light stimulation. Here, we present our early progress towards this goal.

For anatomy, we have acquired a serial section electron-microscopy dataset of the adult xenopus laevis outer retina (collab. Sharm Knecht and Rachel Wong, Washington University, Seattle), and we are now in the process of identifying and tracing double cones and their postsynaptic connections.

In parallel, we are starting to map basic light driven responses from xenopus retinal ganglion cells, for example to steps of light with different spectral composition and temporal profile. Moreover, we aim at establishing high-spatial resolution receptive fields, in the hope that we will be able to spatially isolate the sparse input from individual double cones to the receptive fields of some retinal ganglion cells. If successful, this should allow us to computationally infer which types of visual circuits are strongly driven by double cones, and how their signals are integrated with the signals from other photoreceptor types.

Poster Session 1 | Poster Wall 250 | Label: PS1.250

Category: Vision and photoreception

Specifics of optic flow perception in harbor seals (Phoca vitulina)

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In harbor seals (Phoca vitulina), it was said that vision plays a minor role in gathering information underwater due to limitations caused by low light levels and turbidity. However, when moving through turbid water, radial optic flow is elicited. In 2014, it was demonstrated that harbor seals have the ability to detect deviations from a simulated heading in a radial optic flow environment under water. Besides radial optic flow, harbor seals might perceive planar optic flow, which is induced for example when swimming over the seabed or at the water surface. In our study, we investigated whether harbor seals are able to detect their heading from the just mentioned optic flow environments. In a two-alternative-forced-choice experiment, three harbor seals were asked to indicate the position of the focus of expansion (FOE), indicative of the heading of the simulated forward movement, by touching the response target, corresponding to the side of the monitor on which the FOE was presented. The harbor seals were able to determine the simulated heading in the horizontal plane with an accuracy, defined as the smallest detectable angular difference between FOE and the center of the monitor, of 4.96 ± 0.74 deg for planar and 4.61 ± 0.56 deg for radial optic flow. Thus, contrary to previous thoughts, harbor seals have access to optic information under numerous conditions. Optic flow information could be used for various tasks, such as heading detection, estimating traveled distance or predicting time to collision. Ongoing projects aim to unveil specifics of underwater optic flow perception. One hypothesis tested is whether harbor seals can deal with lamellar optic flow better than for example terrestrial animals. Lamellar optic flow is e.g. caused by a drift of particles due to ocean currents. As harbor seals frequently experience lamellar flow in their natural habitat, strategies to cope with it might be present.

Poster Session 1 | Poster Wall 251 | Label: PS1.251

Category: Vision and photoreception

Evolution of vision in sturgeons: opsin genes, photoreceptors and how to see without rod cells in the retina

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Vision is a crucial sense in many animals. Opsin proteins are core components of vision at molecular level. There are five vertebrate visual opsin gene types: LWS, RH1, RH2, SWS1, SWS2. Sturgeons represent an ancient vertebrate lineage and underwent several whole genome duplication events (WGDs) on the top of two common vertebrate WGDs. These duplications affected also the visual opsin genes. In this study we investigated several sturgeon species, representing lineages affected by different WGDs. We focus on retina transcriptomics and we identified opsin genes and their relative expression level. We study sterlet (Acipenser ruthenus), Siberian sturgeon (Acipenser baerii), their interspecific hybrids, and specimens with manipulated ploidy level. In most sturgeon species, rod opsins (RH1) overwhelmingly dominate their opsin genes expression profiles, which is common in vertebrates. Of the cone opsin genes, the long (LWS) and shortwave (SWS2) sensitive opsin genes dominate the expression profile. Unlike many other fishes, there is low to no expression of the middle-sensitive RH2 opsin. In starry sturgeon (Acipenser stellatus) we found complete absence of the rod opsin (RH1) expression, and the RH1 gene in this species show frameshift mutation towards its C-end. The rod transduction cascade is also missing, and the histology suggests only cones in the retina. Hence, this species most likely lost the rods entirely. We investigated this in A. stellatus and its hybrids with A. ruthenus, both in several life stages. Interestingly, the expression of the pseudogenized RH1 has been resurrected in the hybdrids. We further performed the analysis of retina morphology (photoreceptor cells, ganglion cells) in A. ruthenus, A. baerii and A. stellatus and the application of FISH (fluorescence in situ hybridization) application in A. stellatus and A. ruthenus to better understand evolution of vision in this enigmatic fish group.

Poster Session 1 | Poster Wall 252 | Label: PS1.252

Category: Vision and photoreception

Mechanism shaping the sensitivity of red photoreceptors in jewel beetles (Buprestidae)

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Across the animal kingdom, visual sensitivity to red wavelengths (>600 nm) is relatively rare. In insects, the ancestral trichromatic visual system was comprised of UV, blue and green photoreceptors, but red photoreceptors have evolved independently in some groups (e.g. butterflies, dragonflies, bees and beetles). For several of these groups, we do not know the mechanism that shapes the spectral sensitivity of red photoreceptors, which limits our understanding of visual processing and evolutionary processes. Here, we use anatomical and physiological studies to investigate the retina in two buprestid beetles, Chalcophora mariana and Coraebus undatus. Both species have four photoreceptor types – UV, blue, green and red – and the red photoreceptor peaks at 600 nm. Previous research indicates that buprestid beetles possess an opsin, peaking at 570-580 nm, and that further tuning to longer wavelengths must be achieved via other mechanisms. Some insects use screening pigments to shift photoreceptor sensitivity, however, we did not identify screening pigments in anatomical sections. Alternatively, photoreceptor opponency may influence photoreceptor sensitivity. Using intracellular recordings, we find evidence for a red-green opponent channel that can account for the difference between opsin absorbance and photoreceptor sensitivity. Our results uncover the likely mechanism that shapes red photoreceptor sensitivity in buprestid beetles and suggest that these beetles may be able to discriminate long wavelength colours.

Poster Session 1 | Poster Wall 253 | Label: PS1.253

Category: Vision and photoreception

Vision in Motion: Unravelling the visual capabilities of Australian strobe ants, Opisthopsis spp.

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Vision is crucial for ants' daily activities, including navigation and predator avoidance. Ants have adapted their eyes' morphology and physiology to suit their lifestyle and environment. Strobe ants of the Australasian genus Opisthopsis are notable for their eyes uniquely positioned towards the posterior region of the head and their distinctive 'strobing' locomotion consisting of bursts of acceleration interspersed with short pauses. This study examined the adaptations of the visual systems of two common species, O. rufithorax and O. haddoni. We characterised the external structure of their compound eyes (n=5 per species) and found a similar number of ommatidia (O. rufithorax, 745±14; O. haddoni, 769±20) and facet diameters (16.9±0.7; 16.9±0.3 μm), typical of day-active ants. Mapping the distribution of the facet sizes, we found that the largest facets concentrated in the dorso-frontal region, suggesting an adaptation for detecting visual stimuli from above. Using electroretinography, we measured flicker fusion frequency (FFF) to assess their ability to see rapid movements, finding the FFF values were 174±14 Hz for O. rufithorax (n=5) and 139±9 Hz in O. haddoni (n=5), comparable to other fast-moving diurnal ants (e.g. Myrmecia croslandi, 188.7 Hz; Oecophylla smaragdina, 132 Hz). Additionally, the spatial resolving power and contrast sensitivity were evaluated through electroretinography to understand their visual acuity further. Combined with our field observations, these findings highlight strobe ant's unique morphological and behavioral traits for predator avoidance and navigation in complex environments. Our study compares the visual systems of these species within their ecological context, shedding light on the intricate relationship between eye anatomy and ant behavior.

Poster Session 1 | Poster Wall 254 | Label: PS1.254

Category: Vision and photoreception

A versatile multi-colour spatial visual stimulus projector for the analysis of colour processing

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For the analysis of colour processing, a visual stimulation system is required that offers high spatio-temporal resolution, precise synchronization with physiological equipment, and that is spectrally adapted to the research animal's photoreceptor sensitivities. Commercial displays and projectors, however, are optimized for human vision, are restricted to the use of three colour channels in the visible range, and rarely offer synchronization. Here, we present a versatile visual stimulus projector for the analysis of combined spatial, temporal and spectral response properties and that is adaptable to most visual systems. It is based on a digital light processing module with up to five colour channels and provides more than 100 Hz multi colour frame rate, high spatial resolution, precise synchronization with a two-photon laser scanning microscope for "fly back" stimulation, and customizable colour combinations. We have adapted this system to suit the analysis of visual processing in Drosophila. For this purpose, we developed a stimulus battery, covering a wide range of full-field and spatially structured stimuli. With this toolset, we are able to extract multiple response properties, including chromatic and achromatic spatio-temporal receptive fields of visual neurons. Together, our optophysiological approach provides the means to gain further insight into the neural computations underlying colour processing in Drosophila and many other organisms, and to elucidate the contribution of different cell types and circuit mechanisms to colour vision.

Poster Session 1 | Poster Wall 255 | Label: PS1.255

Category: Vision and photoreception

Regionalization and spectral tuning of an opponent photoreceptor class in the retina of Heliconius melpomene, a Nymphalid butterfly

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The availability of light in a habitat, combined with the evolutionary plasticity of insect compound eyes led to a plethora of solutions for efficient neural processing. The compound eye is the prime substrate for functional adaptations on different organizational levels, from molecular to anatomical. We have recently shown that nymphalids possess blue and green (BG) -sensitive photoreceptors with red (R) opponency, located within the ommatidia at the position R1&2 that is usually reserved for UV or blue (B) sensitive photoreceptors. An additional red screening pigment is present next to the rhabdom that shapes the sensitivity of the R unit. We investigated the spatial distribution of the BG+R- photoreceptor class in the eyes of Heliconius melpomene. Intracellular recordings showed a pronounced variability in the B and G peaks of spectral sensitivities in these cells across the retina. Regionalization was revealed also via optical measurements of eyeshine colour and spectral sensitivity of the ommatidial pupils. Serial light microscopic sections were made to measure the distribution of the red screening pigments across and along the depth of the retina. The sections were fixated with gaseous aldehydes instead of liquid, to preserve the pigments. Intracellular recordings with a motorized, positionable goniometer were made for a precise measurement of spectral sensitivities at known locations relative to the animal. Using the combination of sensitivity and natural illuminant spectra, we show that the spatial variation of spectral sensitivity in BG+R- leads to the equalization of receptor excitation across the light field gradient. The fine-tuned receptor class appears to be a spectral matched filter that performs as a hard-wired white balance-adjusting mechanism.

Poster Session 1 | Poster Wall 256 | Label: PS1.256

Category: Vision and photoreception

The Visual Systems of Crab Spiders and Running Crab Spiders: Convergent or Conserved?

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Visual systems act as a crucial interface between animals and their environment, and are often subject to a combination of strong selective pressures and morphological and functional constraints. As a result, convergent evolution is a common theme in animal vision. Spiders possess a modular visual system consisting of four eye pairs representing two separate visual pathways: a pair of primary eyes, and three pairs of secondary eyes. The arrangement and orientations of these eye pairs are highly variable among spiders, and have important functional implications as they directly impact the visual field. Despite over 100 million years of divergence, the families Thomisidae (crab spiders) and Philodromidae (running crab spiders) exhibit many remarkable morphological and ecological similarities, including flattened bodies, widely-separated eyes, well-developed visual neuropils and diurnal visually guided hunting behaviours. However, whether the visual systems in the two families are functionally similar, and if so, whether they are convergently evolved or ancestrally similar remains largely unknown. We used geometric morphometrics to compare the position and orientation of eyes in 65 spider species based on synchrotron micro-computed tomography. We show that the visual systems of the two families are similar but not identical, and have evolved convergently from their predicted shared ancestral state to have laterally shifted posterior lateral eyes, likely allowing these ambush hunters to detect moving objects in the periphery. Additionally, philodromids show a medial shift of the anterior eyes, presumably helping them visually pursue their prey. Further, we quantified the extent of disparity, modularity and integration across our dataset to demonstrate that the eye pairs coevolve to a large degree, while maintaining a small degree of semi-independence, which might help spiders adapt their eye arrangement and orientations in response to their morphologies and/or ecologies

Poster Session 1 | Poster Wall 257 | Label: PS1.257

Category: Vision and photoreception

How to keep an arthropod eye focused during rapid growth

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One of the most important features of any image-forming eye is correct focusing, which relies on tight coordination between lens optics and the spacing between the lens and retina. In vertebrates and in squid, it is known that underlying processes involve a combination of genetic regulation and visual feedback. Although related processes remain relatively elusive in arthropods, our research group has collected evidence that suggests that in arthropods passive regulatory processes are particularly important. Here, we examine the principal camera-type eyes of larvae of the sunburst diving beetle, Thermonectus marmoratus as they undergo a phase of post-embryonic rapid growth and optical readjustments while transitioning from second to third instar stages. For spacing between the lens and retina, these changes take place within less than an h and we find evidence for osmoregulation playing an important role. Specifically, we find that swelling of support cells which are situated in that region, correlates with natural shifts in haemolymph osmotic pressure. Moreover, as expected, interfering with this process leads to farsightedness. Changes to the lens take slightly longer, within a few hours. To investigate changes in the lens, we use RNAi to knock down a widely expressed cuticular lens protein (Lens3). Interestingly, this does not lead to significant changes in focal length, but results in the formation of 'cataracts', causing the projection of blurry, low contrast images with no systematic shift in focus. This is unlike vertebrates, in which reduced image contrast results in the dysregulation of eye growth, causing refractive errors such as myopia. These results further support that arthropods may not require visual input to establish correctly focused eyes. Taken together, our findings point towards these larval camera-type eyes being an informative biological model to explore the poorly understood etiology of refractive development in arthropods.

Poster Session II

Poster Session 2 | Poster Wall 1 | Label: PS2.001

Category: Learning, memory and cognition

Cognitive abilities of barn owls: what we know and what we don't know

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Cognition refers to flexible abilities of animals beyond basic behaviors. Animals exhibit cognitive behaviors at multiple levels of complexity. The question arises which species does reach which level of complexity and how is it driven by evolution, life style, brain size or brain organization.

Birds possess many cognitive abilities. We are interested in cognition in barn owls, nocturnal raptors that hunt in a solitary manner using both visual and auditory information. Each hunt is costly. Thus, owls need to carefully manage energy use. Early studies showed that barn owls are proficient in discriminating and localizing sounds. Our objective here is to assess more recent experiments from our laboratories and others, along with circumstantial evidence, to elucidate cognitive abilities in barn owls. Notably, visual-search and attention capabilities have been shown in barn owls that resemble mechanisms in humans. Moreover, experiments showed that owls – after hearing a first sound – do not approach a possible prey right away but wait for a second sound, thereby confirming the validity of the first sound. Additionally, in the field as well as under laboratory-controlled conditions, barn owls develop individual foraging strategies. Preliminary analysis also suggests the barn owls are capable to memorize the environment and self-navigate in complete darkness.

Circumstantial evidence suggests that owls can discriminate sounds made by different individuals, and that they are treating handlers differently. Particularly, owls are less stressed when a familiar trainer is handling. Likewise, observations in the field suggest that the owls might possess episodic memory, demonstrated by an owl that was caught in one location and avoided that location for several days after. These observations, however, need more in depth validation through quantitative experiments.

We propose that barn owls can serve as an effective model for the evolution and mechanisms of cognition.

Poster Session 2 | Poster Wall 2 | Label: PS2.002

Category: Learning, memory and cognition

Size-selective mortality alters brain-size, cognitive executive functions and innovative behavior in zebrafish, Danio rerio

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Human and non-human predators target individuals of specific size-classes in fish populations. While larger fish are captured in most fisheries, small fish fall prey to gape-limited fish predators. Increased mortality of larger fish often fosters the evolution of fast life-history emphasizing early reproduction at smaller size, i.e. higher energy investment into reproduction. This could be traded-off with lowered energy investment into other energy-expensive organs like brain. This may lead to reduced brain-size & cognitive abilities. As evolution of a fast-life history typically also comes along with changes in personality traits like boldness, cognitive ability could also be affected through an evolutionary adaptation in behaviours. We test this using three experimental evolution-generated selection lines of zebrafish, (Danio rerio) adapted to large, random & small size-selective harvesting over five generations. We assessed the potential for divergent selection responses in relative brain-size, cognition related executive functions like inhibitory ability (using a detour task) and working memory (using an object-permanence test), and problem-solving ability in individual fish. We found that the large line had significantly larger relative brain-size but there were no changes in the small line compared to controls. The small line fish showed significantly reduced inhibitory learning ability and working memory potential, while the large line fish solved the problem quicker compared to the controls. Our results show that size-selective mortality can lead to evolutionary changes in brain-size and cognitive functions in fish.

Poster Session 2 | Poster Wall 3 | Label: PS2.003

Category: Learning, memory and cognition

Neuronal modulation after associative and non-associative learning on antennal lobe and mushroom body output level

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The familiarity of sensory stimuli strongly influences animal behavior and cognitive processing. Observations in honeybees demonstrate that the differentiation between familiar and novel olfactory stimuli significantly affects learning performance. On a neurophysiological level, it is established that olfactory projection neurons undergo modulation during the process of familiarizing with an odor. However, an unexplored area for investigation involves the potential modulatory effects on higher-order brain areas, particularly at the mushroom body output, known as the center for learning and memory.

In this study, we investigate neuronal modulation in the antennal lobe and mushroom body output pathways following both associative and non-associative learning paradigms. Utilizing extracellular single-unit recordings, we examine how these distinct forms of learning influence the activity patterns within the output regions of the antennal lobe and the mushroom body in honeybees.

Poster Session 2 | Poster Wall 4 | Label: PS2.004

Category: Learning, memory and cognition

Evolution of reward perception and learned behavior in Drosophila

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Animal behavior is shaped by both evolution and individual experience. One important behavior is recognizing appropriate food among various sources and remembering that source for future consumption. We have previously found that Drosophila melanogaster can form better long-term memory of L-arabinose, a natural non-nutritious sugar, compared to its enantiomer D-arabinose. Intriguingly, when given a direct choice between L- and D- arabinose, flies prefer D- over L-arabinose. As L-arabinose in ripening fruits may serve as a food cue for D. melanogaster, it is possible that other Drosophila species, living in different ecological niches with different food habits, may have different responses to D- and L-arabinose. Based on this rationale, we screened 28 divergent Drosophilidae species in a two-choice feeding assay to evaluate their preference for D- vs L-arabinose and used an associative appetitive memory paradigm to evaluate their L- and D-arabinose memory. Among these species, we found that D. mojavensis behaves opposite to D. melanogaster, preferring L- but remembering D-arabinose better. In both species Gr43a receptor is important for long-term memory and substitution of D. melanogaster Gr43a with D. mojavensis Gr43a is sufficient to switch the long-term memory of D- and L- arabinose, without changing the innate preference for these sugars. Dissecting the neuronal circuit and assessing neuronal activity by calcium imaging we found that response of Gr43a expressing neurons in the leg switches upon receptor substitution. These results show that in an existing neuronal circuit, variation in a gustatory receptor can change learned behavior without altering innate behavior and provides a plausible mechanism for evolution of reward percepts and learned behavior.

Poster Session 2 | Poster Wall 5 | Label: PS2.005

Category: Learning, memory and cognition

Pattern recognition in insects: which features do foraging hawkmoths rely on to detect flower patterns?

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Insects exhibit a remarkable ability to locate flowers amidst complex, ever-changing environments, a skill facilitated by visual pattern perception. Foraging insects utilize floral patterns not only to identify but also to memorize and interact with flowers. A key aspect of this interaction is the insects' sensory bias towards certain pattern characteristics, notably a preference for radial patterns observed in many nectar-feeding species. These radial cues are suggested to represent 'nectar guides', floral patterns that lead pollinators to the flower's nectary. Studies have shown that honeybees and bumblebees can recognize these pattern features across variations in size, orientation, or contrast. However, how insect brains, with their limited processing capacity, achieve invariant pattern recognition, remains unknown.

To investigate how nectar-foraging insects recognise flower patterns, we study naive flower choices in the diurnal hawkmoth, Macroglossum stellatarum. Using variations of their innately preferred radial patterns, we dissected local feature preference. Our findings reveal that hummingbird hawkmoths, a solitary pollinator, also exhibit a capacity for invariant pattern recognition. Moreover, our comparison of foraging choices indicated an unexpected preference for radial patterns that contain isolated central elements, as well as disrupted radial features that extend to the flower's edge. Based on these results, we postulate a simple model which could underly their radial pattern recognition, and thus provide insights on the mechanisms underlying pattern recognition in insects.

Poster Session 2 | Poster Wall 6 | Label: PS2.006

Category: Learning, memory and cognition

Hippocampal coding in a social group of wild bats: representation of identity, sex, hierarchy, affiliation, and interactions

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Social animals live in groups and interact volitionally in complex ways. However, little is known about neural responses under such natural conditions. Here we investigated hippocampal CA1 neurons in a mixed-sex group of 5–10 freely behaving wild bats, which lived 24/7 in a laboratory-based cave and formed a stable social network. In-flight, most hippocampal place-cells were socially modulated, and represented the identity and sex of conspecifics. Upon social interactions, neurons represented specific interaction-types. During active-observation, neurons encoded the bat's own position and head-direction, together with the position, direction, and identity of multiple conspecifics. Identity-coding neurons encoded the same bat across contexts. The strength of identity-coding was modulated by sex, hierarchy, and social-affiliation. Thus, hippocampal neurons form a multidimensional socio-spatial representation of the natural world.

Poster Session 2 | Poster Wall 7 | Label: PS2.007

Category: Learning, memory and cognition

Numbers matters: honeybees preferentially use numerical cues in an ecologically relevant task of quantity discrimination

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Despite a growing body of evidence that a wide variety of animals can count objects, the ecological relevance of possessing concepts of number remains questioned in most species, in particular invertebrates. Would individuals still rely on numbers if other - less computatively demanding - cues were available to evaluate quantities? In primates, a numerical bias is present, i.e. individuals tend to preferentially use the number of items to categorize quantities rather than correlating non-numerical cues, such as density or total surface area. In this study, we investigated which cues would be favoured by honeybees during an ecologically relevant task of quantity discrimination. Individual free-flying honeybees were trained to discriminate between two sets of images displaying either two or four dots, to get a sucrose solution. Here, importantly, individuals could either use continuous cues of quantity (ex.: total surface) and/or numerical cues to resolve the task as would be normally the case in real-life scenario. Transfer tests' performances revealed that honeybees preferentially used numerosity rather than surface ratio to choose between quantities. We also looked at the individual strategies of each bee, revealing that 25% of honeybees did use the difference in surface to guide their discrimination suggesting that, despite an overall numerical bias different strategies still exists at the individual level drawing a more complex picture of quantity evaluation and use of numbers in honeybees. Ultimately, these results contribute to strengthen the relevance of numerical cognition as an essential part of honeybees' cognition and highlight the evolutional convergence of the number sense between Vertebrates and Invertebrates.

Poster Session 2 | Poster Wall 8 | Label: PS2.008

Category: Learning, memory and cognition

Analysis of mushroom body synaptic circuits in the adult Apis mellifera brain

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The European honeybee, Apis mellifera, is an important model organism exhibiting exceptional neuronal plasticity evident in high-order sensory integration centres like the mushroom bodies (MBs). The MBs receive information from primary sensory input structures, like the antennal and optic lobes. Within the MB calyx, projection neurons (PNs) of the two input regions form complexes of synaptic contact (microglomeruli, MG) with MB intrinsic neurons, the Kenyon cells (KCs). KCs are classified into two distinct classes based on the morphology of their dendritic specialisations: class I and class II KCs. MG are an important site for synaptic and neuronal plasticity, most significantly during a honeybee's transition from in-hive to outside tasks. The transition is reflected in a reduction in the number and density of MG. At the same time, an increase in the MB calyx volume indicates that KC dendritic branching and outgrowth, and therefore an increase in the number of postsynaptic partners, takes place. The exact MG circuitry and individual KC morphology required for such plasticity remains mostly unexplored. How do class I and II KCs contribute to individual MG? Are there different morphological categories within the two KC classes, and if yes, how do these postsynaptic profiles contribute to a single MG? We used consecutive age cohorts and performed fluorescent dye pressure injections into the vertical lobes of the MBs combined with immunohistochemical approaches to stain small KC populations and label both pre- and postsynaptic MG partners in the MB calyx. Based on 3D reconstructions, the shape and connectivity of KC dendrites were analysed. Our results suggest the existence of different morphological categories of dendritic specialisations within class I and II KCs, which could indicate a significant degree of plasticity at the synaptic level.

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Poster Session 2 | Poster Wall 9 | Label: PS2.009

Category: Learning, memory and cognition

Acoustic wayfinding in flight: probing bat's ability to recall spatial locations using learned auditory cues

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Bats, known for their reliance on sound for orientation in 3D environments, provide powerful research models for investigating the role of auditory cues in both overt and covert spatial attention. Overt attention refers to the orientation of sense organs (eyes, ears, etc.) toward a stimulus, while covert attention refers to the allocation of cognitive resources to a stimulus, without orienting movements. In the present study, Egyptian fruit bats are used to understand auditory attentional processes and their impact on spatial navigation. Four bats are trained in a flight chamber to locate a landing perch in the dark and learn the association between a distinct acoustic cue and the physical location of the perch. We compare flight trajectory, sonar beam direction, and sonar click rate between valid trials containing reliable cues that accurately signal the physical location of a perch and invalid trials containing cues that misdirect the animal to a location without a perch. The directional aim of the bat's sonar beam and the difference in flight time to the perch on valid and invalid cue trials serve as metrics of overt and covert attention, respectively. Extended flight times in invalid cue trials will suggest covert attention to a learned spatial location. This research offers insights into the use of covert and overt attentional processes by Egyptian fruit bats during spatial navigation.

Poster Session 2 | Poster Wall 10 | Label: PS2.010

Category: Learning, memory and cognition

Humans forage in a classic reinforcement learning task

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Because the world changes over time and is only imperfectly observable, many of the decisions we make are necessarily uncertain. How do we navigate such uncertainty? From the perspective of cognitive neuroscience, the classic answer would be that we evaluate the benefits of each potential choice and then choose the one promising the greatest reward, modulo some exploratory noise. However, an ethologist would argue that we would stay with previously rewarding choices until the payout drops below a certain threshold, at which point we start exploring other options. While both hypotheses wield considerable influence within their respective fields, it remains uncertain which one best describes human decision-making. Here, we asked whether human decision-making was better described as as a compare-alternatives process ("Reinforcement-learning" [RL]) or as compare-to-threshold process ("foraging") in a classic testbed of decision-making under uncertainty from the RL literature: a restless k-armed bandit task. We found that the foraging model was a better fit for participant behavior. This was because it better predicted the participants' tendency to repeat choices (versus switch between options) on both individual and group level. Interestingly, the foraging model was also able to predict the existence of held-out participants with very prolonged repetitive choice runs, a pattern of choice that was almost impossible within the RL model. Together, these results suggest that humans use foraging (compare-to-threshold) computations, over RL (value-comparison) processes, even in a classic reinforcement learning task. These findings highlight the need to integrate ethological insights to build more accurate models of human decision-making processes.

Poster Session 2 | Poster Wall 11 | Label: PS2.011

Category: Learning, memory and cognition

Involvement of the teleost inferior lobe in a problem-solving object manipulation task

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Besides mammals and birds, some species of teleost fishes like wrasses also possess relatively large brains and are capable of complex behaviors such as tool use. Such behaviors require the ability to manipulate objects in a precise and goal-directed way.

Our previous work has demonstrated that in teleost species like wrasses and cichlids, the telencephalon and inferior lobe (IL, a ventral structure composed of hypothalamic and mesencephalic cell populations) are enlarged compared to other species, and are abundantly connected together. The IL has evolved particularly in teleosts, and has no homolog in the tetrapod brain. Given that the IL receives multisensory inputs and mainly projects to the cerebellum, we hypothesized that this structure could be involved in the fine motor control required for tool use.

We developed a protocol of Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) using an ultra-high field magnet (17.2T) to visualize neuronal activity in the brain of the cichlid Amatitlania nigrofasciata during a goal-directed object manipulation task. Fish were trained to open a simple puzzle box by pulling on a pivoting lid in order to reach food. Then, their brain activity was assessed using MEMRI. Results show a significant increase in neuronal activity of the IL and telencephalon in the experimental group compared to the control group (fed without object manipulation), while no significant difference in the activity of other brain regions was found.

Our results show the involvement of the IL, a non-telencephalic structure, in a goal-directed object manipulation task.

Overall, our results raise the possibility that non-telencephalic structures may perform pallial-like functions and sustain complex cognition in teleost brains.

Poster Session 2 | Poster Wall 12 | Label: PS2.012

Category: Learning, memory and cognition

Learning from occurrence and termination of reinforcement in Drosophila

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Much research revolves around learning from the occurrence of a reinforcement, while less attention is paid to learning from termination of it. However, receiving a punishment not only feels bad, but its termination brings relief and also feels good. These experiences result in aversive and appetitive learning of associated cues, respectively. In turn, receiving a reward vs. the frustration when it is terminated support appetitive and aversive learning. Our work has established such timing-dependent valence-reversal as an across-species principle (Gerber et al. 2019). We report on our ongoing experiments, in Drosophila melanogaster as a study case, understanding the mechanisms and implications of timing-dependent valence reversal with focus on the contribution of the dopamine system, and on the role of the cAMP cascade in this respect.

References: Gerber et al. (2019). Timing-dependent valence reversal: a principle of reinforcement processing and its possible implications. Curr Opin Behav Sci, 26, 114-120.

Poster Session 2 | Poster Wall 13 | Label: PS2.013

Category: Learning, memory and cognition

Assessing the effect of ultradian light exposure on learning and memory in adult female mice

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The process of memory formation is complex and has been uncovered in both vertebrates and invertebrates. This ability to learn and memorize salient stimuli is vital for any animal as it has direct consequences on survival. Specifically in mammals, memory consolidation can have sex specific differences depending on the stimulus. Thereby making it pertinent to study both sexes in experiments that assess direct effects of context specific stimuli on cognition. One such stimulus is the ultradian light cycle T7(3.5hr L: 3.5hr D), the exposure to which causes significant cognitive deficits in mice, within just 2 weeks compared to a circadian cycle T24 (12hr L: 12hr D). However, these effects have only been uncovered in adult male mice, leaving the females completely in the dark.

The objective of this study was to assess the effects of T7 exposure on the cognitive abilities of adult female mice. To do so, we subjected adult wild type female mice (3–5-month-old) to T7 for 2 weeks and assessed the effect on their cognitive abilities compared to females reared in T24. Both groups were tested for a) long term memory by a novel object recognition test and b) working memory by a Y-maze test. Further, the same animals were exposed to 2 more weeks of T7/ T24, and their spatial memory was tested with a Barnes maze test. The results for all the behavioral tests suggest cognitive deficits in females and indicate similarities to the cognitive deficits that were observed in males in this context. These results further reinforce the importance of an animals light environment and how it can affect key physiological processes.

Poster Session 2 | Poster Wall 14 | Label: PS2.014

Category: Learning, memory and cognition

How experience modulates performance across taxa: a comparative study on visual perceptual learning in bees and humans

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Our brain is plastic: with dedicated training, we can develop expertise or recover cognitive abilities lost e.g., following a stroke. This exceptional property of our brains is shared by other species. For example, mice raised in a stimulating environment rich in new objects to explore and in social interactions will develop greater cognitive test-solving abilities while delaying the effect of aging. But is it universal? Can such cognitive plasticity be observed in an insect, the honeybee? Although the bee exhibits sophisticated learning and cognitive abilities, its short lifespan and lack of neurogenesis in the adult stage could hinder the possibility of improving its performance with experience. With this project, we investigate visual perceptual learning in bees, namely improvement of visual discrimination capacities with intensive experience. In humans, intensive training – generally over several weeks and with thousands of trials – on a task of detection or discrimination of a given stimulus (e.g., detection of a given oriented bar), allows to obtain stable finer discrimination capacities over time. This perceptual learning phenomenon could be explained by synaptic reorganization, an increase in dedicated receptors and/or attentional phenomena. However, this improvement is often very specific, task dependent. A spontaneous transfer to novel tasks may nevertheless take place if a variety of perceptual tasks are used during training. We show here results from subjects from both species extensively (~6 training hours) trained to discriminate low-level visual stimuli (colors, orientations...) under similar conditions. Indeed, we also wished to confirm in humans perceptual learning abilities and properties with shorter training durations compatible with bees experiments. Some subjects were trained on a single task while other were confronted to three different tasks. The subjects were then subjected to novel visual tasks to test for transfer possibilities.

Poster Session 2 | Poster Wall 15 | Label: PS2.015

Category: Learning, memory and cognition

Uncovering associative learning-induced changes in locust olfaction through in-vivo neural recordings and machine learning

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Associative learning enables the ability to generalize information and influence behavior from the reinforcement of our experiences. This powerful adaptive learning mechanism is found throughout biology with varying capabilities. While some biological systems require multiple experiences to learn, insects can generalize information from very few interactions. In the presence of environmental distractors and over great distances, insects can distinguish odors to seek out food and detect predators. The biological ability to generalize a stimulus over different concentrations and contexts has yet to be reproduced through engineered systems. Here, we used in-vivo recordings and machine learning to investigate the neural computations behind olfactory generalized learning in locusts from only a few presentation examples.

A trained odor was presented at different concentrations along with untrained distracting odors. Electrophysiology recordings were obtained from the antenna lobe (AL) of locusts during odor presentation while simultaneously recording palp opening behavior. Using AL recordings from naive and trained locusts, we identified learning-induced changes in in-vivo neuronal responses. Comparing palp responses, we analyzed how neural learning changes guide locust behavior. Additionally, we utilized a recurrent neural network (RNN) mimicking the excitatory and inhibitory neurons in the AL and trained via backpropagation to emulate the neural responses observed in both naive and trained locusts. Imposing biologically plausible constraints on the network's connectivity structure, we achieved models that accurately represent the locust AL neural circuitry. Through analysis of weight adjustments within the RNN network, we identify specific changes that elucidate the learning mechanisms employed by the locust during learning tasks. These results guide the understanding of fundamental learning principles that can be applied to various biological and engineering systems.

Poster Session 2 | Poster Wall 16 | Label: PS2.016

Category: Learning, memory and cognition

Adaptive innate preferences of solitary generalists and its flexibility

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any solitary generalist insects must locate food sources soon after emergence. This necessitates an innate search template which is specific enough to discriminate objects, yet general enough to allow for the diversity of objects relevant as food to that species. Understanding how the tiny brains of insects encode such a template innately is of interest to both ecologists and neuroscientists. We used the solitary generalist pollinator Eristalinus aeneus to understand how the innate food search template can arise through a small number of sensory cues spanning multiple sensory modalities. Using field and laboratory behavioral assays and electrophysiology, we found that the innate floral choices of the hoverfly Eristalinus aeneus are a product of contextual integration of broad plant-based olfactory cues and visual cues with high spectral intensity in the 500-700 nm range and radial symmetry. Such a template is parsimonious yet does not reject the broad ranges of food options available to a hoverfly. We also found that hoverflies can exhibit complete extinction of this innate attraction to the floral cues, and can retain this aversive learning for several days. Conversely, they can quickly learn to associate neutral floral objects with food, and lose this appetitive learning within 48-72 hours. This is starkly different than social insect pollinator that continue foraging on suboptimal resources because of social facilitation. We posit that such understanding can help us derive better strategies for pollinator conservation in the face of a rapidly changing world. Finally, based on this innate floral template, we developed an image classifier, and found that an image classifier built on hoverfly's innate floral template is great at rejecting non-flowers (90% accuracy) and very good (85% accuracy) at identifying flowers. But most importantly, it is extremely quick at these tasks. So, understanding insect search strategies can inspire efficient solutions.

Poster Session 2 | Poster Wall 17 | Label: PS2.017

Category: Learning, memory and cognition

Effects of sleep-like quiescent state on memory consolidation in the pond snail Lymnaea

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We hypothesized that a sleep-like quiescent state enhances memory consolidation in the pond snail Lymnaea staganalis. We performed the EBSL (escape behavior suppression learning) experiment on the snails. In this experiment, snails are first placed on a petri dish lid with an external aversive stimulus, KCl solution. When learning training begins, the snails spontaneously escape from the petri dish lid, but their escape is suppressed by the external aversive stimulus. Once learning is established, the escape behavior is suppressed even when there is distilled water outside the dish during the test session. During the experiment, the number of escapes and the latency to the first escape are measured during training and test sessions. In the present study, during the 3-hour intermission between the training and test sessions, we divided two groups: one group that spontaneously experienced sleep-like quiescent state by being allowed to move freely, and the other group that was inhibited from experiencing a quiescent state by momentary stimulation with a sucrose solution. The results showed that there was no significant difference in the number of escapes between the training and test sessions in the group that experienced the quiescent state, and the latency to the first escape was longer in the test. In the group that was kept active, the number of escapes increased during the test compared to the training, and the latency was not significantly different. These results suggested that a sleep-like quiescence state enhanced memory formation during EBSL in Lymnaea.

Poster Session 2 | Poster Wall 18 | Label: PS2.018

Category: Learning, memory and cognition

From connectomic to behavioral complexity in larval Drosophila?

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Larval Drosophila are an established model system for many questions in the behavioural neurosciences, including associative learning and memory (Thum and Gerber 2019). Recently, the dense electron microscope reconstruction of the memory center of the larval brain (Eichler et al. 2017) has revealed that more than half of the types of chemical-synapse connections had previously escaped attention, a result confirmed in adult flies (Takemura et al. 2017). This unexpected connectomic complexity begs the question whether a corresponding complexity can be uncovered in the complexity of learning capabilities in these animals. In this context, we investigate whether larvae show hallmarks of cognition-like associative processing, namely sensory preconditioning, second-order conditioning, and conditioned inhibition in odour-taste types of learning assays.

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Poster Session 2 | Poster Wall 19 | Label: PS2.019

Category: Learning, memory and cognition

Brain that changes itself: changes in the brain and behavior along the bumblebee queen life cycle

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Throughout the life cycles of the Bumblebee (Bombus terrestris) queens, their behavior undergoes significant changes. These transformations involve alternating increases and decreases in exposure to light, transitioning from foraging in the field to residing in complete darkness within the colony. Despite these observable changes, the exact adaptation of their nervous system to these shifts remains unclear. To address this, we conducted MRI scanning on bumblebee queens at two stages: before diapause and after diapause, using diffusion tensor imaging (DTI) method to scan the bees. We show here for the first time that brain areas, including the visual lobes and the mushroom bodies undergo changes in their conductivity properties during the bumblebee queen life cycle. These results, along with additional behavioral and physiological examinations, provide compelling evidence of the profound neurobiological changes taking place in the insect brain throughout their life cycle. These adaptations are likely pivotal in facilitating insects' adjustments to their diverse lifestyles at different developmental stages.

Poster Session 2 | Poster Wall 20 | Label: PS2.020

Category: Learning, memory and cognition

Constructing quantitative ethograms of walking bumblebees during free exploration to test for effects of common pesticides

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Bumblebees (Bombus terrestris) are central place forages with remarkable learning and memory abilities. They have been shown to use path-integration-based vectors to navigate their environment, as well as being able to commit those vectors to short- and long-term memory. In their role as a pollinator, bumblebees come in frequent contact with pesticides, which leads to various motor and sensory defects, while the cognitive effects of pesticides are understudied and much less clear. To this aim, we have developed a novel behavioural assay which allows for rigorous testing of the mechanisms underlying free exploration behaviour, as well as testing the effects of common pesticides on them. The assay consists of a large behavioural arena, where bees can be filmed at high resolution with a novel lock-on tracking system. All relevant movements can then be tracked using machine learning algorithms, which allows for further characterisation of movement types, behavioural motifs, and the transition likelihood between those motifs. Such an extensive quantitative ethogram has the advantage of unbiasing behavioural analysis and unravelling the subtle differences between control groups and those exposed to pesticides.

Poster Session 2 | Poster Wall 21 | Label: PS2.021

Category: Learning, memory and cognition

Flexible use of colour and social cues in the giant honeybee, Apis dorsata

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Honeybees and bumblebees utilise social information to locate rewarding flowers and enhance foraging efficiency. Social cues can increase the attractiveness of flowers to bees, facilitating the rapid association of floral traits with rewards. The utilisation of such information is also enhanced by experience, highlighting its significance in the foraging ecology of social bees. Colour is an important floral cue and bees show spontaneous and learned preferences for colour. Since social information is available to bees foraging on flowers along with other sensory information, it is of interest to understand how bees weigh social information and colour during flower choice.

In an experimental arena, we investigated how wild Asian giant honey bee (Apis dorsata) foragers weigh social information and colour. We found that bees spontaneously prefer feeders with live or dummy bees over feeders without such social cues. After training to a neutral stimulus, (UV-reflecting grey), foragers spontaneously preferred blue over yellow stimuli during tests. Subsequently, three sets of bees were each trained to either a blue, cyan, or orange stimulus containing 30% sucrose solution as reward. During unrewarded tests, each trained bee was presented with a choice between a feeder of the trained colour and an uncoloured feeder with four dummy bees. The blue-trained bees consistently chose the bee feeder, cyan-trained bees randomly chose the cyan feeder and the uncoloured feeder with dummies, and the orange-trained bees preferred the uncoloured feeder with dummies significantly. The cyan stimulus provided the highest colour contrast against the background, while orange provided the lowest. Our findings demonstrate that the reliance on social cues is contextual, varying with the salience of the colour presented. Such flexibility in using sensory and social cues can facilitate exploration and the discovery of novel resources by bees foraging in nature.

Poster Session 2 | Poster Wall 22 | Label: PS2.022

Category: Learning, memory and cognition

Cuing effects on praying mantis strikes are long-lasting and disparity dependent

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In primates, prior cuing of spatial attention affects the perception of a subsequent target, depending on the properties of the cue. The influence of cue properties on insect visual behaviour is however largely unexplored. In this study, we investigated how the stereoscopic disparity of a cue influences mantis predatory strikes to subsequent targets, and how long the effect of a cue lasts. We made use of a previous paradigm showing that mantises only strike at localised (elementary) motion if it is preceded by wide-field (figure) motion. To investigate the influence of disparity, we fit mantises with 3D glasses and presented them with a 3D elementary motion target either without a prior cue, or preceded by a figure motion cue displayed with stereoscopic disparity (in 3D) or monocularly. In a separate experiment, we also varied the duration between a 3D figure motion cue and the elementary motion target between 2 and 50 seconds. Mantises were most likely to strike at the elementary motion target when the figure motion cue was in 3D simulating a cue 2.5 cm from the mantis. Mantises did, however, still strike significantly more when the cue was displayed monocularly compared to when it was absent. Mantises were also just as likely to strike at the elementary motion target even 12 seconds after the figure motion cue. Their strike probability decreased after 12 seconds but reached close to zero only 50 seconds after the figure motion cue. Our results thus suggest a role for sustained spatial attention in the predatory responses of mantis.

Poster Session 2 | Poster Wall 23 | Label: PS2.023

Category: Learning, memory and cognition

Neural mechanisms of learned sociability in Drosophila

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Sociability refers to an animal's ease of living among members of its own species. This trait is a prerequisite for all other ethologically important social interactions and collective behaviors. Despite an appreciation of the importance of sociability, the degree to which it is innate versus learned, and its underlying neural substrates remain unknown. To address this gap, we investigated sociability in the adult fly, Drosophila melanogaster. Group-raised female flies are undisturbed by one another, come into close proximity and engage in tactile interactions. By contrast, animals raised individually produce a constellation of fearful reactions toward other conspecifics. Remarkably, single-housed animals can learn to be sociable over the course of several hours in the presence of other flies: avoidance behaviors diminish and are replaced by interactions observed among group-housed animals. Thus, fear of conspecifics appears to be a default state and sociability is learned through interactions with other animals. Using environmental and genetic manipulations we found that sociability learning requires olfaction but not vision, taste, or touch. A neural silencing screen of 195 brain cell types revealed that specific cell types from the ?/? and ?'/?' lobes of the mushroom body subserve both initial fearful reactions as well as learned sociability. Cell types found in our screen have direct synaptic connections, suggesting a mechanistic model for sociability learning. We tested this model further by recording neural activity in mushroom body circuits during inter-fly interactions over several hours. Our results reveal that sociability toward conspecifics is learned continuously rather than innate and requires subcircuits within a prominent associative learning and memory center in the fly.

Poster Session 2 | Poster Wall 24 | Label: PS2.024

Category: Learning, memory and cognition

Mouse lockbox: a sequential mechanical decision-making task to investigate complex mouse behavior

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Recent advances in automated tracking tools have sparked a growing interest in studying naturalistic behavior. Yet, traditional decision-making tasks remain the norm for assessing learning behavior in neuroscience. Here, we present an alternative sequential decision-making task to study complex mouse behavior. We developed two different 3D-printed mechanical puzzles, so-called lockboxes, that require a sequence of four steps to be solved in a specific order. During the task, the mice move around freely, enabling the emergence of complex behavioral patterns. We observed that mice exhibit a high level of motivation, willingly engage in the task, and learn to solve it in only a few dozen trials.

To analyze the strategy the mice use to solve the task, we used three cameras to capture different perspectives and developed a custom data analysis pipeline. The pipeline allows the automated detection of interactions of the mice with the different lockbox parts for a large corpus of video material (>300h, 12 mice).

We find that an increasing number of mice are capable of solving the lockbox task across trials. The mice are significantly more engaged with the lockbox for trials in which the task is solved and they furthermore express a higher-than-random preference towards the state-advancing lockbox parts. Preliminary analyses suggest that the increased solving capability is not due to an increased interaction time with the task, but potentially due to low-level motor learning and/or due to learning of a high-level solution strategy.

Although our data analysis is preliminary, we find that freely moving mice can rapidly learn to solve complex, multi-step mechanical puzzles that are more challenging than most standard tasks. We believe that this task provides a promising balance between natural behavior and a well-defined task that provides anchor points for the analysis of both the behavior and – in future – neural recordings.

Poster Session 2 | Poster Wall 25 | Label: PS2.025

Category: Learning, memory and cognition

Symmetry concept formation in a California sea lion (Zalophus californianus)

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California sea lions live in an environment with constantly changing stimuli. Abstracting and generalizing from these stimuli can lead to concept formation, which promotes cognitive economy and increases the chance of survival. In this study, the ability of California sea lions to form the absolute class concept "symmetry" was tested. The stimuli were black shapes on a white background. A pair of stimuli was referred to as a problem and consisted of a vertically symmetric stimulus and an asymmetric stimulus. In a behavioral experiment, a sea lion was trained to select the symmetric stimulus according to a two-alternative forced choice procedure. The study was divided into 3 distinct phases of acquisition and testing. In the one-problem phase, the animal had to become familiar with the experimental procedure and the discrimination task. Each session consisted of just one problem and a new problem was tested only after the animal had reached a predetermined learning criterion. In total, 30 different problems of increasing complexity were tested. Overall, performance of each new problem started at chance level and increased over time. The number of trials to reach the learning criterion did not gradually decrease with problems. Each problem was learned independently, so performance is stimulus specific. In the six-trials phase, sessions consisted of five different problems, each presented six times. New problems were introduced regardless of the animal's performance. Therefore, the opportunities to learn specific stimulus properties were limited to six trials for each problem. Nevertheless, analyses of our data (inter- and intra-problem learning) show progressive improvement in performance, which suggests the animal developed a symmetry learning set. In the final one-trial phase, two transfer sessions with a total of 60 trial-specific problems were conducted in which the sea lion showed highly significant performance, demonstrating the formation of a symmetry concept.

Poster Session 2 | Poster Wall 26 | Label: PS2.026

Category: Learning, memory and cognition

Know Thy Neighbor: The Neural Basis of Reciprocity in a Highly Social Cichlid

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Cooperation is found across a wide variety of species but presents an interesting evolutionary problem as cooperators incur a cost when helping other individuals. One mechanism for the evolution of cooperation is reciprocity, where individuals reciprocate the previous behaviors of their partner after a time delay. This means that individuals must consider information about their social partner and the likelihood they will receive help in the future, which may require sophisticated cognitive mechanisms for assessing, evaluating, and responding to a variety of social cues. However, few studies have empirically tested reciprocity. Further, the neural mechanisms underlying cooperation and reciprocity are virtually unknown. Here, we used the highly social African cichlid fish, Astatotilapia burtoni, to quantify behavior and circulating hormones in a novel reciprocity paradigm. We then employed whole mount in situ hybridization chain reaction to examine neural activity patterns in response to reciprocal cooperation or a simulated defection. We show that A. burtoni males show direct reciprocity within two hours of receiving assistance or defection from a neighbor. Further, we identify neural activity patterns using immediate early genes in both cooperating and non-cooperating neighbors, as well as the role of dopaminergic signaling in this behavioral context. This work will increase our understanding of social decision-making and deepen our understanding of cooperation across species.

Poster Session 2 | Poster Wall 27 | Label: PS2.027

Category: Learning, memory and cognition

Uncovering the neuro-ethological components of emotions in honeybees.

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The idea that invertebrates may have subjective, internal states assimilable to ‘emotions’ is growing, although their characterization is still at its infancy. We used a multicomponent approach, including behavioral, neurophysiological, and cognitive analyses to determine whether honey bees (Apis mellifera) may experience an ‘emotional state’ of fear upon the perception of an immediate or future nociceptive stimulus. To answer this question, we used a behavioral paradigm sharing distinctive features with the protocol of fear conditioning used in rodents. Bees were trained to associate a contextual blue light with the occurrence of electric shock in an enclosed chamber, which allowed us to characterize the behavioral and physiological building blocks of ‘fear’. Not only did the bees learn and remember that blue light anticipated the electric shock one hour after the training, but their behavior was modulated by the intensity of the shock so that bees trained with higher intensities exhibited higher latencies to approach the blue light. Moreover, bees that learned the association had a different respiratory dynamic than control bees. Furthermore, using HPLC mass spectrometry, we found higher levels of 5-HT (serotonin) in the brains of bees having learned the aversive association compared to control bees. While 5-HT has been shown to underlie several aversive responses in invertebrates, here we show that changes were restricted to learners and not found in controls that had also experienced the shock. This result shows that 5-HT variation was not a mere response to the shocks but reflected a state triggered by the anticipation of shock, akin to anticipatory fear. Serotonin is known for its involvement in mammalian emotions thus highlighting possible similarities between the neural bases of invertebrate and vertebrate subjective states. Our investigation is particularly relevant in the ongoing debate about the presence of sentience in invertebrates.

Poster Session 2 | Poster Wall 28 | Label: PS2.028

Category: Learning, memory and cognition

Vocal production in the Egyptian fruit-bat

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Some species have evolved the ability to use the sense of hearing to modify existing vocalizations, or even create new ones. This ability corresponds to various forms of vocal production learning that are all possessed by humans, and independently displayed by distantly related vertebrates. Among mammals, only a few species would possess such vocal learning abilities. Yet the anatomical, neurophysiological or genetic specificities that determine the ability of a mammal for vocal learning remain largely elusive. With a multidisciplinary approach spanning vocal behavior, anatomy, neurophysiology, and genomics, we explored the vocal learning trait in Egyptian fruit-bats (Rousettus aegyptiacus). First, we tested the necessity of an intact auditory system for the development of this bat typical vocal repertoire. Eliminating pups' sense of hearing at birth and assessing its effects on vocal production in adulthood, enabled us to both causally test the vocal learning ability of Egyptian fruit-bats, and discern learned from innate aspects of their vocalizations. Second, we tested and found evidence for the long standing hypothesis of a neuro-anatomical specialization in vocal learners: a direct projection from the motor cortex to the motoneurons that directly control the muscles of the phonation organ. Third, we investigated what could be the role of cortical neurons in this region that directly projects to laryngeal motoneurons. Finally, we leveraged the identification of this anatomically specialized region of motor cortex to investigate any gene expression that would be specific to the motor cortex of mammal vocal learners. We found that vocal production is sexually dimorphic in the Egyptian fruit-bat and that only some vocalizations need auditory feedback for production. The oro-facial motor cortex forms a specialized circuit for vocal production and is characterized by a core set of non-coding DNA regions, associated with autism, that are under selective pressure.

Poster Session 2 | Poster Wall 29 | Label: PS2.029

Category: Learning, memory and cognition

Statistical learning in honey bee

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The extraction of the statistical properties of complex scenes is a cognitive challenge encountered by different species. Notably, in the visual and auditory domains, the ability to extract probabilistic information from sensory inputs has been shown – among others – in humans and non-human primates, birds, and bees. Such a comparative approach underscores the universality of statistical learning across species and sensory modalities. Here, we adopted the honey bee as an animal model because of its perceptual richness and complex cognitive abilities and investigated its aptitude to detect the probabilistic structure of a sequence of olfactory stimuli. Employing the olfactory conditioning paradigm of the proboscis extension reflex, we showed that bees can discriminate and learn the order of two adjacent, consecutive olfactory stimuli (e.g., AB vs BA). One-hour memory retention tests against the elemental components of the learned sequences showed that bees respond preferentially to the odorant that was temporally associated with the sucrose reward. However, when tested against the rewarded and unrewarded sequences, bees display a selective response towards the conditioned stimulus configuration, indicating their ability to incorporate probabilistic information of a complex olfactory input. This study shows that the ability to extract, learn, and use statistical information from sensory inputs is a fundamental cognitive toolkit that can also be found in insect brains.

Poster Session 2 | Poster Wall 30 | Label: PS2.030

Category: Learning, memory and cognition

Learning your instincts: Dissecting nest-building behavior in Lamprologus ocellatus, a shell-dwelling cichlid from Lake Tanganyika

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Nest-building behavior has evolved across many animal taxa – from termites to spiders and from birds to the great apes. But the neurogenetic underpinnings of animal architecture have remained obscure. We are investigating nest building in Lamprologus ocellatus (LO), a shell-dwelling cichlid fish from Lake Tanganyika. LO build nests by manipulating empty snail shells, inserting them vertically into the substrate, and covering them with sand. We showed that they are doing this by executing a sequence of stereotyped behavioral motifs.

We next asked if nest-building behavior is genetically programmed or learned. To answer this question, animals were reared without physical or visual access to shells. When exposed to an empty shell for the first time, these shell-naïve fish hesitantly engaged with it and built a nest rather laboriously. Eventually, they succeeded and built nests in species-typical fashion, stringing together the same behavioral motifs as experienced fish. Following this first clumsy attempt, shell-naïve individuals learned fast, and their following nest-building activity resembled experienced animals in duration and dexterity. Visual exposure to building animals without the opportunity to physically manipulate a shell did not shorten the learning time of shell-naïve animals. Likewise, physical interactions with a shell that was suspended in the tank, but unavailable for nest building, did not accelerate the learning process and thus could not substitute for the real experience. Fascinatingly, a single building opportunity in an otherwise shell-deprived animal was sufficient to “imprint” a capacity for normal nest building several months later, suggesting the formation of a long-term memory by a single trial. Using immediate-early gene mapping, we have begun to localize the brain regions activated during nest building. In summary, we propose that nest-building behavior in LO constitutes an innate program that is unfolded through one-trial motor learning.

Poster Session 2 | Poster Wall 31 | Label: PS2.031

Category: Learning, memory and cognition

Knockout in zebrafish reveals the role of glucocorticoid receptor in lateralisation and learning

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Research in mammals has demonstrated that glucocorticoids (GCs) regulate many physiological processes, ultimately affecting a number of traits including behavioural and cognitive ones. In this study, we attempted to dissect the contribution of the main GCs pathway related to stress, which involves the GCs Receptors (Gr), on cognition. As a model, we used a mutant zebrafish lacking Gr (gr-/-).

We first focused on cerebral lateralisation, the differential cognitive processing in the brain hemispheres, which is extremely variable among individuals potentially because of stress responsiveness. We characterized the lateralization phenotype of gr-/- zebrafish using two on the processing of visual stimuli and one test based on motorial in absence of visual stimuli. Gr loss affected lateralization but only in the visual test based on the response to the social stimulus. This finding was supported by a follow up experiment on the natural variability of gr expression in wild-type zebrafish. Individuals with higher mesencephalic expression of gr in one hemisphere were more likely to use that hemisphere to process a social stimulus, while individuals with more lateralised telencephalic gr expression were more likely to have stronger lateralisation.

We further investigated GRs' effects on cognition using two learning tasks (visual and spatial learning) and one cognitive flexibility task (spatial reversal learning). We found evidence that in all the learning tasks gr-/- took longer to reach the criterion, suggesting learning impairments. Moreover, in the spatial reversal learning task, the loss of GR determined a slower decrease in the number of errors across days.

We conclude that gr is involved in all the cognitive processes investigated in fish, suggesting a potential widespread effect also in other vertebrate groups.

Poster Session 2 | Poster Wall 32 | Label: PS2.032

Category: Learning, memory and cognition

Behavioral state influences encoding mechanisms of color and odor integration in a model insect, the bumblebee Bombus impatiens

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[Note to chair/s – this work crosses boundaries of listed categories, including 'learning, memory and cognition', 'Olfaction...', 'Vision and...' and potentially 'Spatial Orientation and Navigation'. I selected the category that made sense to me, but am happy to be considered in any of the above]

Bumblebees rely on diverse sensory information to locate flowers while foraging. The majority of research exploring the relationship between visual and olfactory floral cues is performed at local spatial scales and applicable to understanding floral selection. Floral cue-use during search remains underexplored. This study investigates how the bumblebee Bombus impatiens uses visual versus olfactory information from flowers across behavioral states and spatial scales. At local spatial scales, non-flying animals in an associative learning paradigm will access learned multimodal cues in an elemental fashion – where a single component of the cue is capable of eliciting responses. However, bumblebees flying in a windtunnel shift cue-use strategy depending on the spatiotemporal scale of cue encounter. When both color and odor cues mimic local/ within patch spatial scale, bumblebees exhibit a gradient of elemental responses – with highest responses to the complete multimodal color+odor cue, followed by intact color, then intact odor. When cues mimic an intermediate/ between patch spatial scale, bumblebees exhibit configural responses to the learned cue – with high responses to only the learned color+odor cue. Thus the underlying physiological state, sensitive to both activity level and spatiotemporal scale of sensory information, is modulating encoding and utilization of multimodal floral cues.

Poster Session 2 | Poster Wall 33 | Label: PS2.033

Category: Learning, memory and cognition

Statistical Learning and Chunking of Complex Visual Scenes in Honeybees (Apis mellifera)

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In the recent years, honeybees have demonstrated impressive learning skills in a variety of domains and have proven to be a relevant model to study the cognitive system of invertebrates. One area in which they excel is visual learning, and particularly in statistical learning tasks. Notably, they have been shown to detect complex regularities in multielement visual scenes and to be able to group up to three elements as a single memory unit – a chunk. Chunking is a crucial cognitive, mostly implicit, process. It has been widely observed in vertebrates – including humans – and allows them to compress information and bypass memory limits during learning. However, chunks themselves have been systematically shown to have limits in the number of items that they can comprise. The present study deals with honeybees' ability to form chunks larger than 3 items. Using an incidental visual statistical learning paradigm, we investigated how free flying honeybees spontaneously encode multielement visual scenes and their ability to form 4-item chunks. The results inform us about the constraints of honeybees' memory system and allows us to draw comparisons between their cognitive abilities and other animals' cognition, including humans.

Poster Session 2 | Poster Wall 34 | Label: PS2.034

Category: Learning, memory and cognition

Saccopteryx bilineata: A Promising Candidate for Exploring Neuronal Substrates of Mammalian Vocal Learning

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The Greater sac-winged bat Saccopteryx bilineata is a unique mammal with remarkable features, including vocal learning, vocal practice, and song. The learning and practice of syllables are manifested in the production of long multisyllabic vocal sequences, called pup babbling. This behavioural readout of learning processes offers the opportunity to explore underlying neuromolecular mechanisms. I present my current project, which explores the neuronal substrates of vocal learning in Saccopteryx bilineata. Specifically, I aim to investigate the functional role of speech-relevant genes, such as FoxP2, during vocal learning. I hypothesize that I will observe an upregulation of FoxP2 during babbling (i.e. ongoing vocal learning processes) compared to my control groups (i.e. silence and innate vocalizations). Furthermore, I am examining the expression of immediate early genes to map neuronal activity during vocal production, practice and learning. To accomplish this project, I have successfully implemented a synergy of lab and field methods by combining behavioural observations and acoustic recordings of wild pups with subsequent harvesting of brains in the field.

Currently, I am analyzing the expression of the different genes with the method of free-floating in-situ hybridization. The ultimate goal of this project is to investigate speech-relevant genes in a vocal learning mammal and establish connections between findings from songbirds and humans.

Poster Session 2 | Poster Wall 35 | Label: PS2.035

Category: Learning, memory and cognition

Learning through sight and smell: Correlates of bimodal sensory integration in Drosophila

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Insects show remarkable abilities to sense multimodal stimuli and form memories associated with them . This acquired information with updated values to existing cues can be essential at a later point in time. Conventional conditioning experiments report strong learning ability in vinegar flies (Drosophila melanogaster) using olfactory cues. In our study, we established a T-maze choice assay that combines the presentation of both visual and olfactory stimuli in an aversive conditioning paradigm to study the effect of bimodal integration on learning performance. We show that the presence of additional information during training differently affects the strength of learning for the individual olfactory and visual components. Further physiological investigation in the D. melanogaster model using two-photon microscopy and calcium sensors aided in understanding the role of higher brain regions such as the mushroom bodies and the lateral horn, especially communication between the different centres, in multimodal information processing. We also utilized our learning paradigm to compare and contrast the behaviors of diverse members of the genus Drosophila and report robust differences in their ability to employ visual and olfactory stimuli in cognitive tasks. These results help pave the road for future investigation on more nuanced behaviors arising from simultaneous information processing in insects and how sensory experience shapes cognitive performance.

Poster Session 2 | Poster Wall 36 | Label: PS2.036

Category: Learning, memory and cognition

Individual strategies in a challenging ordinal learning and rotation task

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What is a small-brained animal to do when the task they encounter is hard to solve? When a complex task is presented, individuals may have different strategies for solving it. Such strategies could range from successfully learning the difficult task, to finding tactics that work frequently, to opting out of learning the task in favour of a simpler strategy.

We trained honeybees (Apis mellifera) to select an ordinal position from an array of six positions along either a horizontal or vertical line to receive a reward of sugar water. Four groups of bees were trained to differing ordinal positions (2nd vs. 3rd position) and orientations (horizontal or vertical). Following training, bees were tested on their ability to extrapolate the ordinal position at a different orientation. For example, bees which had learnt to visit the 2nd option along a horizontal line were tested on choosing the 2nd position along a vertical orientation.

Interestingly, all bees did not solve the problem in a similar way. Instead, multiple strategies were observed for performing the task at both group and individual levels. Some bees were able to learn the initial task, and subsequently perform a rotation extrapolation. Other bees employed alternative strategies during the tests. For example, some bees preferred to land at the edge, then sample sequentially along the line. In colonies with many individuals, a diversity of search strategies may be optimal to enable new options as flower resources change during the day, and thus avoid myopic over-exploitation of one foraging option.

The results provide insight into how and why animals may succeed or fail at certain tasks depending on the difficulty and alternative strategies available. This is particularly interesting to study in miniature bee brains, as such strategies have applications to bio-inspired problem solving using minimum neural circuitry.

Poster Session 2 | Poster Wall 37 | Label: PS2.037

Category: Learning, memory and cognition

Visual rule based cognitive flexibility in bumblebees

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Bees in the wild must forage for flower nectar in an ever-changing environment, where the amount of nectar reward from a given flower species can change dynamically over the course of a day. Cognitive flexibility – the capacity to adapt one's behaviour in response to environmental change – is a necessary survival skill for animals and is often considered to be a hallmark of their intelligence. Decades of research has shown that bees quickly associate visual cues with a sucrose reward and learn rules that allow them to categorise multiple cues based on shared properties. However, it is still unknown to what extent they can flexibly switch between such rules.

To test whether bees demonstrate visual rule based cognitive flexibility, we devised a task inspired by the "Wisconsin Card Sorting Test" – a standardised task to test cognitive flexibility in humans. We opted for a multiple-choice paradigm, where freely flying bumblebees chose from eight rewarded or unrewarded feeders to land on, each associated with a visual cue (coloured shapes) displayed on a vertical high-refresh rate monitor. The bees were trained to categorise these visual cues, based on either their colour or shape in a block-based trial structure. Once they achieved 100% correct landing choices, they were presented with a novel stimulus set to test whether they could generalise across the relevant cue dimension, before moving on to the next rule block. Motivated forager bees completed this task involving learning of four rules and three rule-switches within a day.

We observed that individual bees differ significantly in their learning rate after a rule-switch, largely due to variations in their perseverative behaviour. By reconstructing trajectories of their flight, we are currently investigating how their flight patterns relate to differences in their learning rate, and its potential relationship to active vision mechanisms.

Poster Session 2 | Poster Wall 38 | Label: PS2.038

Category: Learning, memory and cognition

A working memory assay in larval zebrafish

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In many behavioral contexts, the brain relies on working memory to temporarily retain information in a form that can be processed and used to assist future decision-making. However, it remains unclear how this memory is represented in the brain, and what neural processes entail its formation and deletion.

We have developed a working memory assay in larval zebrafish, in which the larvae use past visual cues to assist them in deciding where to escape when a non-directional acoustic stimulus is presented, after a time delay. We also show that this memory consists not only of an overt component, which is evident during spontaneous swimming but also a longer-lasting covert component that is only revealed when the animal performs a long-latency C-start, a delayed escape that facilitates information integration. Furthermore, we show that the escape behavior is accompanied by a rapid reset of the memory trace.

We take advantage of the optical transparency of the zebrafish larva to uncover brain regions pertaining to the working memory. We performed brain-wide calcium imaging during a head-restrained version of the cue-delay-startle assay, using a volume-scanning light-sheet microscope. We demonstrate that head-restrained fish can perform the behavior, and we map brain areas whose activity correlates with different features of the assay. In particular, we find areas that show persistent activation during the delay period, with a timescale matching that of the working memory, and areas, including the raphe nucleus, in which responses to the acoustic stimulation are modulated by the previous visual cue. Combining these maps with known features of connectivity, we can propose models of the circuit organization of working memory in the larval zebrafish brain.

Our results demonstrate the usefulness of zebrafish larvae as a model for the study of the subcortical brain regions involved in working memory.

Poster Session 2 | Poster Wall 39 | Label: PS2.039

Category: Learning, memory and cognition

Decoy effect in social decision-making in zebrafish (Danio rerio)

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The principles of economic rationality suggest that rational individuals consistently aim to maximize utility when making choices. The axiom of independence of irrelevant alternatives (IIA) posits that the preference order between two options remains unchanged regardless of the presence of other alternatives or decoys in the choice set. However, organisms, ranging from simple slime mold to human beings, often deviate from IIA, especially when confronted with combinations of multiple-choice attributes.

In this poster, I will present novel experiments and results from my investigations into the decoy effect using zebrafish shoaling choices across single and multiple dimensions, individual differences, and the effect of group size on the decoy effect. In the single-dimensional study, we observed sex-based differences where the decoy effect was evident only among males, particularly when presented in a specific order of trichotomous and dichotomous shoal options.

In the two-dimensional study, we manipulated shoaling characteristics using virtual points that mimic fish movement in terms of speed and spread, while presenting the decoy option asymmetrically. Please note that this study is currently in progress, and I will be presenting ongoing experiments. Furthermore, we explored the differential effects of hunger state and interindividual differences in various behavioural tests, including the decoy effect on shoaling preference. We found that hungry fish showed increased interest in investigating decoy options, despite the lack of significant differences in shoaling preferences among individuals.

In our study on group rationality, we report that larger shoaling groups were less susceptible to the decoy effect, suggesting a collective decision-making process that overrides individual biases. Our findings contribute to a deeper understanding of social decision-making processes in a zebrafish neurobiological model system.

Poster Session 2 | Poster Wall 40 | Label: PS2.040

Category: Learning, memory and cognition

The cellular and functional organisation of the dopaminergic teaching signal in Drosophila larvae

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Dopaminergic neurons (DANs) carry out multiple tasks in the brain, including the transmission of information related to rewards and punishments across various animal species. They are responsible for evaluating sensory input, storing resultant associations as memory, and continuously updating them based on their relevance and reliability. Accurate comprehension of the dopaminergic system's operation necessitates an understanding of the specific functions mediated by individual DANs. To this end, our research employs Drosophila larvae, which possess approximately 12,000 neurons in their brains, of which only around 1% are DANs.

The presynaptic projections to the mushroom body (MB) – a brain region pivotal for associative olfactory learning in insects – are limited to only eight larval dopaminergic neurons. These DANs are further subdivided into two clusters: the primary protocerebral anterior medial cluster (pPAM) comprises four cells, and the dorsolateral 1 cluster (DL1) comprises the remaining four cells. Our findings confirm previous research that demonstrates that the pPAM DANs innervating the MB's medial lobe encode for a reward signal. Furthermore, we have identified four DANs in the DL1 cluster - DAN-c1, DAN-d1, DAN-f1, and DAN-g1 - that encode for a different aspect of a punishment signal, with DAN-g1 being of central importance.

To summarize, our investigation has revealed the existence of a cellular division of labor among larval DANs concerning the transmission of dopaminergic reward (pPAM cluster) and punishment signals (DL1 cluster). Individual DANs in each cluster encode for distinct but partially overlapping aspects of the teaching signal. The striking resemblance in the organizing principle of larval DANs with that of its adult counterpart and the mammalian basal ganglion suggests that there may be a limited number of efficient neural circuit solutions available to address more complex cognitive challenges in nature.

Poster Session 2 | Poster Wall 41 | Label: PS2.041

Category: Learning, memory and cognition

Effect of the temporal sequence of stimuli on olfactory associative learning in Apis mellifera

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In natural settings, informative odorants are often mixed with concurrent environmental odors, which can mask the presence of the former. Recognizing the presence of meaningful odorants in this complex environment involves intricate generalization and discrimination processes. Here we investigate the contribution of the temporal order of odorants, as well as the role of sensory adaptation in the identification of meaningful olfactory stimuli. Sensory adaptation is the phenomenon by which an animal's sensitivity to a stimulus decreases after sustained exposure to it. Recent experiments from our lab demonstrated that the ability of honeybees to detect target odorants embedded in complex mixtures is improved when bees are exposed to the masking odorants before conditioning with a mixture that contains both target and masking odorants. These results pointed out a crucial role of sensory adaptation in target odor detection. However, in these experiments, it remained unclear whether this capacity was due to the animals being adapted to the masking odor or to the animals learning that the masking odor predicted no reinforcement. To disambiguate it, we performed olfactory conditioning experiments in which unrewarded exposure to the masking odors was made before or after rewarded trials using as conditioned odor a mixture of the masking and the target odor. We observed that specific learning towards the target odor was improved only when prolonged exposure to the masking odor precedes rewarded trials with the mixture, while it did not when unrewarded exposures were made immediately after the rewarded trial. These observations led to subsequent experiments aiming to demonstrate the animal's ability to distinguish among different temporal orders of the odorants. So far, our results support the idea that olfactory working memory and sensory adaptation serves as fundamental mechanisms to sharpen olfactory discrimination.

Poster Session 2 | Poster Wall 42 | Label: PS2.042

Category: Learning, memory and cognition

How can the Octopus vertical lobe help in revealing fundamental principles of associative learning networks?

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The discovery of activity-dependent long-term potentiation (LTP) in the vertical lobe (VL) of Octopus has indicated that the octopus VL and mammalian hippocampus share not only anatomical features but also the cellular process believed to mediate learning and memory, thus suggesting the independent evolution of similar cellular mechanism. However, the VL LTP induction was found to be NMDA-independent and presynaptically expressed - two properties that are inconsistent with the canonical associative Hebbian LTP in the hippocampal CA1 region. Here, we integrate results that show that LTP expression and maintenance are mediated by persistent activation of NOS in the postsynaptic interneurons and that LTP expression is mediated through NO retrogradely facilitating presynaptic transmitter release (Stern-Mentch et al. 2022; Turchetti-Maia et al. 2018). We will suggest that mechanistically, the NO system in the VL provides an alternative solution to that of the NMDA receptor in the hippocampus. The connectome results (Biedel & Meirovitch et al. 2023) contributed morphological support to this idea as they show that the majority of the amacrine interneurons (SAMs) receive only a single synaptic input. This monosynaptic connection undergoes LTP, suggesting that functionally, a SAM is analogous to the hippocampal dendritic spine. The connectome results revealed intense reciprocal connections between the SAMs and serotonergic processes. Such interconnection resembles the 5-HT-mediated heterosynaptic facilitation of the sensory-to-motoneuron synapse in the defensive reflex of Aplysia, and indeed, preliminary results suggest that 5-HT-dependent PKC cascade, which in Aplysia mediates 5-HT-induced synaptic dishabituation, is used in the VL to mediate the activity-dependent LTP induction. This comparative overview supports the idea that the evolution of cognitive abilities in advanced mollusks has converged to fundamental properties shared by associative learning networks.

Poster Session 2 | Poster Wall 43 | Label: PS2.043

Category: Learning, memory and cognition

Behavioural Flexibility During Waiting Periods

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Many animals can associate sensory cues with rewards even if separated by long temporal intervals (trace conditioning). However, behavioral flexibility during the intervening interval has rarely been studied. Here we show that fruit flies can form odor-reward associations with up to minutes of temporal distance. Once trained, flies stayed where they experienced the future-reward predicting odor even after odor removal. In contrast, they did not stay after odor removal if they expected immediate reward.

To study the flexibility of this behavior we trained flies with two odors, each rewarded at a different time after odor presentation. We found that they can associate individual odors with different reward delivery times and adjust their odor approach timing accordingly. During testing, if both reward-paired odors are presented in different parts of the olfactory arena and flies are allowed to move between them freely, they first move to the odor that has been paired with immediate reward and later move to the location of the odor paired with future reward, at a time that matches the trained reward delivery interval (40s after odor presentation).

By presenting a third unpaired odor immediately after the two paired odors, we show that flies postpone their visit to the second paired odor until after the unpaired odor removal. Therefore, flies exhibit their response only under the appropriate temporal and olfactory context, demonstrating context-dependent behavioral flexibility.

Through cell-type-specific optogenetic experiments, we found that dopaminergic neurons projecting to mushroom body compartments γ4 and γ5 most effectively induce trace conditioning memory. Blocking these and corresponding output neurons impairs trace conditioning.

Our results demonstrate that Drosophila can order the sequence of navigational behaviors according to the expected timing of rewards and open the way to a mechanistic understanding of the underlying neural substrate.

Poster Session 2 | Poster Wall 44 | Label: PS2.044

Category: Learning, memory and cognition

Investigating emotion-like behavior in cuttlefish Sepia pharaonis

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In recent years, there has been a notable increase in interest in exploring emotion-like states in invertebrates. Cephalopods, particularly cuttlefish, are renowned for their intricate brains and advanced cognitive behaviors among invertebrates. Nevertheless, there remains a lack of research dedicated to investigating whether cuttlefish experience emotions.
Here we developed a judgement bias test based on the concept and paradigm used in bumblebee research in 2016 (Solvi et al. 2016). Through conducting associative training using black circle and black square patterns as positive and negative stimuli respectively, our aim was to examine whether there were any significant differences in responses to ambiguous stimuli between the control and pre-reward groups among cuttlefish. The ambiguous stimuli were patterns whose shapes were transitions between black circle and black square. Due to limitations in animal availability, our preliminary results indicated that cuttlefish were successfully trained in a five-tunnel apparatus. Animal tracking was utilized to analyze the cuttlefish's routes and head orientations during testing trials. Additionally, pattern switching trials suggested that cuttlefish may prioritize location stimuli, potentially utilizing visual cues as place markers. We anticipate to gather more data in coming months, and this will enable us to explore deeper into cuttlefish's emotion-like state. The present study thereby promotes animal welfare and offers fresh insights into emotional differences across phyla. Additionally, this research has the potential to offer new perspectives on how emotions evolve in diverse species in the future.

Poster Session 2 | Poster Wall 45 | Label: PS2.045

Category: Learning, memory and cognition

Behavioral Consequences of Mushroom Body Medial Lobe Malformation in Drosophila melanogaster

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The accurate wiring of neuronal circuits during development is the prerequisite for errorless and functional control of behavioral output. In Drosophila, larvae and adult animals occupy different ecological niches and display largely different behaviors that correspond to their ecological needs. Whereas the neuronal circuits underlying behavioral control in either larvae or adult Drosophila are extensively investigated, our knowledge about the transition of the neuronal network from larval to adult circuit architecture is scarce. The distinctive architecture of the Drosophila mushroom body provides a valuable model system for studying the transition from larval to adult neuronal circuits according to the animals' life cycle. During early metamorphosis the bi-furcating larval γ -lobe Kenyon cells are pruned back and subsequently extend their long protrusions along the medial lobe of the mushroom body where the Kenyon cells axons connect with specific modulatory and output neurons in a spatially organized manner, resulting in five distinct compartments. The proper extension of Kenyon cell axons to the medial tips of the adult mushroom body depends on the immunoglobulin superfamily protein Dpr12 that is strongly enriched in the distal γ 4/5 compartments. These compartments are heavily innervated by dopaminergic neurons and have been associated with the control of certain behaviors that are present in larval and adult Drosophila e.g., appetitive short term memory formation as well as exclusive adult specific behaviors such as control of spontaneous horizontal walking behavior or courtship learning and sleep. Here, we show how a spatially restricted defective development of mushroom body circuits drastically alters behavioral control in adult Drosophila.

Poster Session 2 | Poster Wall 46 | Label: PS2.046

Category: Motor systems, sensorimotor integration, and behavior

Descending control of walking direction in Drosophila

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To get from A to B, animals need to control the speed and direction of locomotion. A critical bottleneck in the neuronal circuits controlling walking are descending neurons (DNs), which connect higher-level control centers in the brain to lower-level motor circuits in the ventral nerve cord (VNC) or spinal cord. Here, we analyze how two DN populations contribute to the control of walking direction in Drosophila.

First, we focus on the well-described moonwalker DNs (MDNs). By combining in-vivo patch-clamp recordings in walking Drosophila with antennal mechanosensory stimulation and genetic silencing, we establish that MDNs contribute to antennal touch-mediated backward walking. Each of the four MDNs responds to bilateral antennal touch, and their activity is strongly correlated with backward walking speed, but not turning, in spontaneously walking flies. Hence, MDNs drive backward walking in the context of antennal touch, which could play a role in the negotiation of obstacles in the fly's walking path.

To broaden our understanding of the descending control of walking, we next recorded from DN Meander, which form a population of six DNs. Bilateral Meander activation drives forward walking with a strong turning component, leading to a meandering walking path. Recordings in behaving flies confirmed this: Meander activity is strongly correlated with ipsiversive turning, and weakly correlated with forward speed. Hence, the Meander and MDN populations control different aspects of forward walking, backward walking, and turning.

Finally, we investigated whether the two DN populations controlling walking are also active during flight. Remarkably, both Meander and MDN were strongly inhibited during flight. This flight-dependent gating has the opposite sign to the gating observed in DNs controlling flight-related behaviors and peripheral visual circuits, suggesting that the differential gating of descending pathways enables adaptive behavior in different behavioral states.

Poster Session 2 | Poster Wall 47 | Label: PS2.047

Category: Motor systems, sensorimotor integration, and behavior

Moving to perceive vs moving to achieve: trade-offs in flower approaches of hummingbird hawkmoths

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Visual estimation of distance is core to many insect behaviours – landing, object avoidance, object approach and conspecific approach, to name a few. Since many insects are unable to use stereopsis at longer ranges, they use movement to integrate information on object distances. Object distance can be inferred from movement via two mechanisms: 1) via the object's rate of expansion in the visual field, due to moving towards the object and 2) via the object's rate of displacement in the visual field, due to moving laterally to the object.

How do insects balance moving to gather information and moving to achieve their goal? We consider this question for a hummingbird hawkmoth approaching a flower, where estimating distance to the flower is necessary. The hawkmoths' tortuous approach trajectories, observed in behavioural experiments, may reflect the inherent tension between moving to approach the flower and moving to integrate the information necessary to reach the flower.

We use optimal control theory to generate approach trajectories which balance information gain and fast flower approach. We derive a bound on information gain for both distance estimation models. We find lateral movements provide more information on distance than movements toward the flower. Using our model, we propose that variability across observed approach trajectories arises from changing weightings on information gain and time taken to reach the flower. We combine analysis of hawkmoth trajectories and our model to suggest experiments that might alter the hawkmoths' trajectories by manipulating the distance information provided by an artificial flower.

Our research provides a control theoretic framework to study active sensing in insects and promotes a style of control whereby state estimation is a part of action computation. This is relevant for robotic applications where sensors might require movement not just to complete a task, but to gather information necessary for its completion.

Poster Session 2 | Poster Wall 48 | Label: PS2.048

Category: Motor systems, sensorimotor integration, and behavior

Characterization of central brain neurons controlling adaptive walking in Drosophila

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Animals need to flexibly adjust their walking behavior to efficiently negotiate complex, dynamic environments. Although they are capable of flying, Drosophila spend a large proportion of their active periods walking. In flies, environmental sensory cues are integrated by higher-level brain interneurons (BNs) and conveyed to lower-level motor centers in the ventral nerve cord via descending neurons (DNs). DNs dynamically modulate ongoing motor activity to match the task at hand. The precise organization of these sensorimotor pathways, how BNs are recruited, and how they engage different DN populations for different tasks is poorly understood. Here, we combine optogenetics, behavioural analysis, in-vivo recordings, and connectomics to analyze sensorimotor pathways involved in the control of adaptive walking.

First, we performed an optogenetic activation screen of 131 split-Gal4 driver lines targeting different BN types using a free walking assay. We identified five BNs that control key aspects of adaptive walking, including walking initiation, termination, speed, and direction. Other BNs drove more complex behavioral sequences, such as combinations of backward, curve, and forward walking. Silencing experiments revealed that some of the identified BNs are required for specific aspects of adaptive walking. For example, the BN ‘Roadrunner’ initiates walking, controls walking speed, and is required for flies to increase their walking speed upon starvation – a hallmark of foraging. Next, we traced the connectivity of the BNs in a fly brain EM volume to reveal their inputs and outputs to understand the circuit architecture underlying the central control of locomotion. Finally, we performed in-vivo patch clamp recordings in behaving flies to determine which sensory cues the BNs integrate to control adaptive walking. Combining these approaches will enable a mechanistic understanding of central neuronal pathways that control fundamental aspects of adaptive locomotion.

Poster Session 2 | Poster Wall 49 | Label: PS2.049

Category: Motor systems, sensorimotor integration, and behavior

Shared control of reaching and walking in the lateral frontal cortex of freely moving macaques

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Primates evolved highly refined cortical circuitries subserving the abilities to use the forelimb for reaching and grasping objects. These circuitries are usually conceived as radically distinct from spinal cord central pattern generators responsible for more automatic and rhythmic locomotor activities, such as quadrupedal walking. However, when precise limb placement, velocity or direction changes are required to overcome obstacles or rough surfaces, cortical signals become essential. In this study, we investigated the contribution of lateral frontal cortex regions, typically deemed to be responsible of the voluntary control of reaching-grasping actions, to spontaneous walking. We developed a neurobehavioral approach to record single-neuron activity from the left lateral frontal cortex of two freely moving rhesus macaques while filming their behavior with a multicamera system. A substantial fraction of the recorded neurons showed rhythmic firing patterns synchronized with distinct phases of the contralateral forelimb swing movement. Furthermore, we identified single neurons selectively encoding the first, last, or intermediate steps of a walking episode. Head-free electrical microstimulation of sites hosting walking-related neurons elicited forelimb or axio-proximal movements, suggesting a joint contribution of the lateral frontal cortex to the control of distal and proximal components of walking. Interestingly, neural dynamics during walking partly overlapped with those observed during goal-directed reaching actions, suggesting the existence of a partially shared neural substrate for these behaviors. Our findings demonstrate the joint contribution of the lateral frontal cortex to reaching-grasping and locomotor actions, providing direct support to earlier hypothesis on the phylogenetic origin of skilled reaching from neuronal substrate originally devoted to the voluntary control of locomotion in challenging environments.

Poster Session 2 | Poster Wall 50 | Label: PS2.050

Category: Motor systems, sensorimotor integration, and behavior

Trade-offs between pitch and tempo matching in counter-singing nightingales

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Nightingales are renowned for their large song repertoire, including the acoustically distinct type of so-called whistle songs. These contain temporal sequences of unmodulated tonal syllables, known as whistle syllables, defining the fundamental frequency of the song i.e. the pitch. While recent research has shown nightingales’ ability to adjust acoustic structure to match pitch, the flexibility of their temporal structure remains unexplored. We show that nightingales exhibit a consistent rhythmic pattern in their whistle songs, with a shift in pitch distribution toward lower frequencies as tempo increases. We analyzed whistle songs of naturally singing nightingales and defined tempo as the temporal distance between the onset of whistle syllables. We found whistle songs had a consistent rhythms across a trimodal distribution of tempos, categorizing whistle songs’ tempi into fast (500 ms). Nightingales possess a broader pitch range at slower tempos, while higher tempos correlate with a shift towards lower frequencies. We reconstructed whistle syllables capturing the observed natural temporal and spectral dynamics using a model that describes syringeal dynamics in songbirds. This allows us to understand the underlying physical constraints during whistle syllable production. Building on these findings and previous pitch-matching experiments, we conducted fieldwork to investigate spectral-temporal trade-offs in whistle songs. To this end, we generated synthetic whistle songs that challenge the nightingales’ matching capabilities. Preliminary results show that nightingales can adjust their responses to spectral, temporal or both features simultaneously, offering insights into the optimization strategy guiding their vocal behavior.

Poster Session 2 | Poster Wall 51 | Label: PS2.051

Category: Motor systems, sensorimotor integration, and behavior

Beyond Locomotion: Motor Control of a Sonic Spinal Circuit

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Spinal motor networks exhibit remarkable adaptability, enabling behaviors beyond locomotion, such as sonic signaling or social displays. One such system is the piranhas' sonic motor system, which generates complex acoustic signals for social communication. These sounds are produced by rapid contractions of a single pair of muscles, causing vibrations in the swim bladder. The neurons innervating the sonic muscles are located in the spinal cord. Since locomotor and sonic behavior are activated independently from each other, we hypothesized that separate control regions in the mid- or hindbrain project to these respective spinal circuits.

To identify the control regions commanding these two behaviors, we backfilled neurons connected to cord regions a) innervating sonic and locomotor and b) mediating only locomotor behaviors. To identify the cord regions that contain the sonic motoneurons, we first retrogradely labeled the motoneurons via neural tracer injections in the sonic muscles.

By applying neural tracers in sonic/locomotor segments, we identified several descending neuronal populations known to be associated with motor behaviors, including the Mesencephalic Locomotor Region in the hindbrain, along with other reticulospinal neurons, and the Nucleus of the Medial longitudinal fasciculus in the midbrain. Preliminary tracings from more caudal spinal locations that control locomotor behavior only, were characterized by fewer descending neurons. However, this awaits quantitative verification. By overlapping neurons from either spinal cord regions, we expect to find neurons in the sonic/locomotor region absent in the locomotor-only regions, thus indicating potential neuronal populations that might control sonic behavior.

Poster Session 2 | Poster Wall 52 | Label: PS2.052

Category: Motor systems, sensorimotor integration, and behavior

Behavioural programs in mini-brains: optomotor response in a miniature insect

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Miniaturization in insects requires a reduction and simplification of physiological systems. An extreme example is the visual system of the one-millimeter western flower thrips Frankliniella occidentalis. Owing to the small number of ommatidia and brain size, we asked the question of whether during miniaturization the animal has lost its vision-mediated control for locomotion, in particular the loss of optomotor response (OMR) behaviors. We thus walked animals in an arena surrounded by a rotating drum with vertical black and white stripes. From the walking path, we estimated OMR dependent on the drum's temporal frequency (ω) and spatial wavelength (λ). Thrips typically walk straight when the pattern is stationary and on a circular path during drum rotation, following the angular direction of the visual stimulus. The turning delay in response to the onset of drum rotation was ~1.0 s. We found that the animal's turning velocity depends on the temporal frequency of visual stimulation, following a bell-shaped curve with a maximum at ~3.5Hz. When increasing spatial wavelength from 6° to 90° at constant ω, the turning response starts to develop at ~30° and peaks at ~60°. The spatial wavelength of the elementary motion detector (EMD) model for maximum turning response is 4 times the facet's interommatidial angle (Φ). The latter angle was estimated in 3-dimensional models reconstructed from µCT scans of the animal's head and amounts to ~17°. Thus, the spacing of visual axes and behavioral response closely coincide (~68° vs. ~60°).

Poster Session 2 | Poster Wall 53 | Label: PS2.053

Category: Motor systems, sensorimotor integration, and behavior

Disentangling Cephalopod Chromatophores Motor Units

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Cephalopods are undeniably the masters of camouflage. They can change 3D texture and colour in a fraction of a second. Cephalopods achieve this using chromatophores, the biological equivalent of pixels on a display. Chromatophores are neuromuscular organs composed of a cellular elastic pigment sack surrounded by a set of radial muscles. Chromatophores are not innervated uniformly. A motor neuron typically innervates several muscles, forming a so-called motor unit, and this muscles can be attached to more than one chromatophore. Reciprocally, chromatophores themselves can each also be innervated by more than a single motor neuron, suggesting the existence overlapping motor unit. Our lab has previously developed a computational pipeline to statistically quantify the expansion state of chromatophores in footage of freely-behaving animals. As chromatophores act under the direct stimulation of motoneuron, this non-invasive approach allows the study of neural signals by proxy. In our current studies we increase the spatial resolution and the temporal range of our analyses, by respectively decomposing the activity of chromatophores in more precise kinematic measurements and following changes in innervations over development. Ultimately, this study will result in the development of a new computational pipeline which goal is to disentangle overlapping motor units and study their development over time.

Poster Session 2 | Poster Wall 54 | Label: PS2.054

Category: Motor systems, sensorimotor integration, and behavior

A bat's-ear view: how sensory information streams govern behavioral transitions in the wild

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Sensorimotor integration is a fundamental process by which self-generated or environmental signals inform and guide ongoing behavior. Echolocating bats rely on auditory input from various sources within a single sensory modality to control motor output and meet behavioral objectives. Specifically, bats dynamically tune their information flow via adjustments to the rate, type and direction of their emissions, in response to changes in received echoes. These parameters directly influence the spatial extent and temporal resolution of their sensory volume. This rapid control of attention subserves the broader goal of detecting prey, tracking evasive maneuvers, and enabling capture. Here, we test the hypothesis that the frog-eating bat Trachops cirrhosus, a gleaner which switches between foraging modes when challenged in the lab, selectively applies this same flexible strategy in the wild, depending on properties of the auditory stream. We use a self-logging sound and motion tag to record both the outgoing calls and incoming weak echoes as well as associated behaviors. With this technology, we can monitor both endogenous and exogenous components of the auditory stream, from the perspective of wild bats as they navigate and hunt within an acoustically rich natural environment. We present the first direct observations of these bats (n=20) performing aerial and ground captures in flight and from perches, applying both echolocation and eavesdropping modes. We further show that these bats are highly efficient predators that achieve capture success rates of up to 100% and regularly spend less time hunting their prey than consuming it. This data set can also provide insight into the sensory factors that influence how much time animals dedicate to either foraging mode and their contributions toward maximizing hunting success. Our results reveal how actively-sensing animals attend and respond to parallel streams of information within a shared sensory modality to govern behavior.

Poster Session 2 | Poster Wall 55 | Label: PS2.055

Category: Motor systems, sensorimotor integration, and behavior

Spinal cord neuron density, morphology, and androgen receptor expression associated with a novel hind limb communication signal in foot-flagging frogs (Staurois parvus)

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When novel communication signals arise, how does the nervous system change to accommodate new behaviors? To address this issue, we study a group of frog species that have recently evolved a novel hind limb communication signal known as “foot flagging.” Our prior work showed that foot flagging is androgen dependent, and that the evolution of foot flagging in multiple unrelated species is accompanied by the evolution of higher androgen hormone sensitivity in the leg muscles. Here, we present data that compares patterns of androgen receptor (AR) expression and neuronal cell density in the lumbar spinal cord, which controls the hind limb, between foot-flagging and non-foot-flagging frog species. We found that AR density varies spatially across the spinal cord but does not differ between foot-flagging and non-foot-flagging frogs. However, the lumbar spinal cord of foot-flagging frogs does have higher neuron density compared to the other species. To assess the functional role of AR in the hind limb neuromotor system, we used flutamide implants to block ARs throughout the entire body for three weeks. We then used Golgi staining to label neurons in the lumbar spinal cord and 3D reconstruction to measure their morphological features. Preliminary results show that our manipulation may influence soma size and dendritic branching of hind limb motoneurons in adult male foot-flagging frogs. Overall, these data suggest AR-mediated maintenance of functional morphology in the spinal cord of foot-flagging frogs, with potential implications for understanding the mechanisms by which selection has shaped the neural circuitry that facilitates the foot flag display.

Poster Session 2 | Poster Wall 56 | Label: PS2.056

Category: Motor systems, sensorimotor integration, and behavior

Vocal muscle phenotype reflects sexual dimorphism in singing behaviour in zebra finches

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Zebra finch vocal behaviour is sexually dimorphic: males sing a stereotyped learned song, while females do not. Songbirds produce sound in a unique vocal organ, the syrinx, which is located at the bifurcation of the trachea into the two bronchi. Two pairs of vocal labia are suspended by a bony skeleton which is surrounded by 8 pairs of muscles. Sound is produced when expiratory air-flow induces self-sustained oscillations in the vocal labia. Syringeal muscles modulate sound by tensing and positioning the vocal labia. Myosin heavy chains (MYHCs) are a major component of the contractile machinery of striated muscle cells and functional heterogeneity can be explained by MYHC content and type.

Avian syringeal muscles are among the fastest known in any vertebrate: in adult male zebra finches, these muscles contract superfast and produce low maximal force. This extreme muscle performance is driven by singing exercise, which led us to hypothesize that muscle performance in females differs from males. To test this hypothesis, we quantified muscle performance as well as myosin heavy chain expression and composition on the RNA and protein level.

We found that female syringeal muscles created higher mass-specific forces and contracted slower compared to males. Males expressed higher amounts of superfast myosin, while females predominantly expressed fast MYHC types which are known to produce higher forces. Additionally, proteins involved in the exercise response of skeletal muscles were more abundant in males compared to females. Taken together, our findings support our hypothesis that syringeal muscle performance is sexually dimorphic. The pronounced expression of genes that are hallmarks for exercise adaptations in males, but not in females expands our previous results that muscle performance in males is driven by exercise and suggests that the sexual dimorphism in muscle properties is as well.

Poster Session 2 | Poster Wall 57 | Label: PS2.057

Category: Motor systems, sensorimotor integration, and behavior

What's a startle response? Novel approaches for classifying diverse reactions to looming stimuli in Drosophila melanogaster

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Widespread phyla produce startle responses – rapid, short latency motor reactions to sudden stimuli, such as flinches, jumps or pauses. It remains unclear why startle responses occur before sustained defensive behaviours like freezing and running are initiated; non-exclusive proposals include protective functions, overwhelming muscle activity to reinitiate behaviours and/or switching attentional states. I hypothesise startle responses interrupt ongoing behaviours, resetting the state of the organism to facilitate initiation of sustained defensive responses. Here we track thousands of flies responding to looming stimuli and observe diverse startle responses which are challenging to classify using standard techniques. We aim to take advantage of the characteristic timing of startle responses to uncover features which will aid its classification. Startle reactions should be enriched during the looming stimulus and in the time period immediately following it (loom period) compared with time windows of the same length distant from the loom (no loom period). To this end, we trained a supervised classifier to detect a looming stimulus based only on the fly's behaviour: we predict that this classifier is detecting looming stimuli using signatures of startle behaviour. To inform our understanding of what constitutes a startle response, we used JAABA (Kabra et al, 2014) to train a classifier using a set of features calculated from the tracked fly body part coordinates. The JAABA classifier is a weighted set of simple rules that can separate "loom" and "no loom" categories across thresholds within each of these features. Specific features that contribute more to the classification have stronger weights, and we can use these to build data-driven hypotheses about what constitutes a startle response. This approach will provide a classification of startle responses from which we can study their function and regulation with the experimental rigour enabled by the Drosophila system.

Poster Session 2 | Poster Wall 58 | Label: PS2.058

Category: Motor systems, sensorimotor integration, and behavior

Subthreshold responses to tutor and bird's own song playback in Area X neurons of juvenile zebra finches

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Zebra finches are vocal learners. They learn to sing by imitating their father in a critical window of 90 days post hatch. The songbird's basal ganglia structure, Area X, is known to be essential for vocal motor refinements. However, its potential role in auditory learning and the underlying neural mechanisms remain unexplored. To this end, we tutored juvenile finches with the same live tutor to ensure exposure to an identical template song. We then quantified the juveniles' song performance in the later phase of the critical period. Some juveniles incorporated new syllables that matched the tutor's syllables even in the later part of the critical period. Others modified the spectral and temporal features of the already incorporated syllables gradually towards a crystallized song. This finding supports previous work demonstrating that juvenile zebra finches exhibit individualized learning strategies despite exposure to a common tutor. To test how Area X encodes the tutor song and compares it with the current song performance, we recorded 91 neurons intracellularly in the late critical period (62-82 days post hatch) across 5 birds while playing back the tutor and the bird's own song. While the firing rate of neurons remained unaffected by either playback, 27/84 neurons showed changes in their ongoing membrane potential dynamics correlated with tutor playback. An additional subpopulation of 17/69 neurons displayed similar changes in response to the bird's own song playback. The observed correlations were independent of the individual song learning progress. While auditory input of the bird's own song or the tutor song can induce subthreshold changes in Area X neural activity of awake-listening juveniles, it does not elicit suprathreshold responses capable of exciting or inhibiting downstream neural circuits. This finding suggests that Area X may not play a direct role in sensory learning of the tutor song but could be primarily involved in motor execution learning.

Poster Session 2 | Poster Wall 59 | Label: PS2.059

Category: Motor systems, sensorimotor integration, and behavior

Basal ganglia-cortical dynamics during sleep in songbirds

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Current data indicate that basal ganglia (BG)-cortical network guide sensorimotor learning and supports the hypothesis of a critical role of sleep in this process. We aim to understand BG-cortical dynamics during sleep and its possible contribution to complex sensorimotor learning. To do so, we study birdsong behavior, a complex form of sensorimotor learning by social imitation of conspecifics. A network of interconnected brain nuclei, including a cortico-BG-thalamo-cortical loop (lMAN-AreaX-DLM), is dedicated to birdsong learning and production. We perform large-scale electrophysiological recordings in freely moving (adults and juveniles) birds using Neuropixel probes. We are currently assessing whether events of coordinated activity within neuronal assemblies of the BG (Area X) and their cortical target (lMAN) occur during sleep by analyzing both local-field potentials (LFP) and spiking activity. Preliminary results show that BG-cortical (AreaX-lMAN) LFP coherence in the low frequency ranges are spatially widespread and modulated by sleep states. Neural firing patterns transition from synchronized to desynchronized burst firing during sleep, with single neurons showing significant coherence with local LFP gamma oscillations. Interestingly, AreaX neurons exhibit firing at negative phases of the high gamma oscillations, whereas LMAN neurons exhibit synchronized population bursts co-occurring with sharp wave-like low delta-frequency oscillations of the LFP. Bursting in LMAN, modulated by sleep state, is indeed followed by peaks on a scale of 100ms in the LFP, which corresponds to the low frequency content of the LFP. Furthermore, we also find significant correlations between LMAN neural firing and the output pallidal neurons of AreaX, suggesting that these pallidal neurons drive bursting in LMAN. We are now testing if these patterns of activity reflect offline neural dynamics underlying vocal learning.

Poster Session 2 | Poster Wall 60 | Label: PS2.060

Category: Motor systems, sensorimotor integration, and behavior

Widespread temporal niche partitioning in an adaptive radiation of cichlid fishes

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The partitioning of ecological niches is a key factor during species diversification in adaptive radiations. However, it is presently unknown if and how adaptive radiations are influenced by temporal niche partitioning, wherein species avoid competition by being active during different time windows. Here, we address this question through profiling temporal activity patterns in the exceptionally diverse fauna of cichlid fishes in Lake Tanganyika. By combining week-long behavioural recordings of over 500 individuals from 60 species with eco-morphological and genomic information, we provide two lines of evidence that temporal niche partitioning facilitated diversification in cichlids. First, cichlid species exhibit all known circadian temporal activity patterns (diurnal, nocturnal, crepuscular, and cathemeral) and display substantial inter-specific variation in daily amounts of locomotion. Second, species with similar eco-morphological niches can occupy distinct temporal niches. Moreover, we identify that shifts between diurnal and nocturnal activity patterns are facilitated by a crepuscular intermediate state. In addition, genome-wide association studies suggest that the genetics underlying activity patterns is complex, with different clades using different genes. Furthermore, outlier SNPs were not associated with core circadian clock genes but with multiple genes implicated in neuronal function. These observations indicate that temporal niche partitioning contributed to adaptive radiation in cichlids and that multiple genetic pathways are associated with the diversity and evolution of circadian activity patterns.

Poster Session 2 | Poster Wall 61 | Label: PS2.061

Category: Motor systems, sensorimotor integration, and behavior

Tactile information guides decision-making in a unique oviposition behaviour

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Oviposition is a fundamental aspect of insect reproduction and thus under major evolutionary selection. Involved decision-making processes (e.g. when and where to oviposit) may be informed through intero- and exteroceptive cues promoting the appropriate transitions along the behavioural sequence. With regards to the evolution of oviposition, the Phasmatodea are an intriguing lineage, given that selective oviposition evolved multiple times independently from an ancestral state of simple egg-dropping. For this ancestral state, we assume that selection pressures on the control of where, when and how to lay an egg are relaxed. This opens up the opportunity to establish a new model system for the evolution and control of selective oviposition in insects, in which comparative analyses on the associated physiology, ecology and behaviour are possible. The exceptionally odd oviposition behaviour of Lobofemora sp. (Clitumninae) is characterized by a unique dorso-anterior bending of the abdomen, by which the animal positions a single egg into a small crack or crevice. Here, we identify key components of this behaviour, namely (i) the tendency to lay eggs in a spatiotemporally clustered fashion, (ii) the emerging stereotypical activity patterns within the sequence of single oviposition events and (iii) the preference for certain oviposition site sizes. Finally, we address morphological traits associated with the behavioural sequence. We propose that antennal cues inform the decision to oviposit and play a certain role in the postural control of this elaborate oviposition behaviour.

Poster Session 2 | Poster Wall 62 | Label: PS2.062

Category: Motor systems, sensorimotor integration, and behavior

The neural control of gait switching in larval zebrafish.

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Animals need to perform a diverse range of behaviours to navigate their environment successfully. Larval zebrafish swim using discrete episodes of propulsion called bouts that can be classified into 13 categories of movement, based on fish position and bout kinematics. We aim to elucidate the mechanism of transition between different movements, particularly in the context of forward swimming, on both a behavioural and neural level. To characterise gait-switching behaviour, larval zebrafish were presented with gratings moving from tail to head at different speeds eliciting forward swimming, known as the optomotor response (OMR). Slow gratings predominantly elicited slow swims, characterised by low tail-beat frequency (TBF), rostral tail-bend amplitude (rTBA), head yaw and undulating pectoral fins. Fast gratings triggered a rapid transition from slow to sustained trains of fast swims, characterised by high TBF, rTBA, head yaw and tucked pectoral fins. To uncover the neural correlates of slow and fast swims and elucidate the population dynamics underlying gait transitions, we recorded activity from genetically labelled neural populations in the brainstem of head-fixed larval zebrafish while they performed the OMR in a closed-loop configuration, utilising a 'SCAPE' oblique light-sheet microscope. Head-fixed fish performed slow and fast bouts with similar bout kinematics to freely-swimming fish, but we also observed movements that included switches in TBF and rTBA within a single bout. We therefore use regression analysis, based on kinematics of individual half-beats within each bout to identify neuronal populations associated with different modes of swimming. Using pan-neuronally labelled fish, we explore neural control signals related to onset, offset and switching between motor patterns. By showing how the brain dynamically selects and switches between two distinct motor outputs, we strive to understand fundamental principles in the supra-spinal control of locomotion.

Poster Session 2 | Poster Wall 63 | Label: PS2.063

Category: Motor systems, sensorimotor integration, and behavior

Visually-driven escape maneuvers by individual fish and fish groups

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Escaping from an attacker is a fundamental task that requires rapid sensory decision-making (flee or not) as well as online sensory-motor control to generate a successful evasion trajectory. However, because predators may attack in a wide variety of settings using a range of different attack strategies, sensory-motor algorithms for generating escape behavior must be not only fast but also flexible. We used a novel experimental system to study escape decision-making and escape sequence construction by solitary fish and fish in groups. In groups, individuals may benefit from collective sensing of predator threats, but they also experience conflict due to the fact that fleeing individuals must both avoid the predator and avoid colliding with one another during high-speed escape maneuvers. We combined visual sensory reconstruction methods with inferential tools from machine learning to reverse-engineer the sensory-motor transformations animals use to control escape maneuvers when they are solitary and when they are in groups. Our results shed light on the transformations by which animals convert incoming visual data to non-trivial behavioral sequences, and the mechanisms by which they resolve conflict between distinct objectives when time is short and the stakes are high.

Poster Session 2 | Poster Wall 64 | Label: PS2.064

Category: Motor systems, sensorimotor integration, and behavior

Sex in the city: Sensory resilience and vulnerability of mating in malaria mosquitoes

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The malaria mosquito, Anopheles gambiae, displays a mating behaviour of remarkable complexity and precision. Within their daily, short-lived (~20 min) mating swarms, male mosquitoes gather in groups of thousands, with only a few dozen females entering the swarm. Males use their sensitive auditory system to identify and locate females by the females' characteristic 'flight tones'. The short swarming periods are linked to specific light and temperature conditions around sunset and require a high degree of behavioural coordination between the sexes. We investigate the role of different light and temperature cycles on mosquito mating success. Examining their ability to mate in complete darkness, or constant light, we observed a stark reduction in insemination rates, especially under constant light conditions. We then conducted experiments using daily temperature cycles where a daytime high of 28C drops to a night-time low of 22C. Here, temperature changes were set as an alternative cue for swarming, in either constant darkness or light. Mating reductions were observed at similar rates to constant 28C experiments, suggesting that temperature cannot replace light as a daily synchronisation cue under the chosen conditions. When testing a different species of disease-transmitting mosquitoes which displays a more adaptable mating behaviour, Aedes aegypti, under the same light conditions, we found mating not to not be affected by either lack of, or constant exposure to light. From our results, parallels could be drawn to the impact of light pollution characteristic of human cities, raising novel questions as to how current and future urbanisation will affect disease transmission dynamics and how vector control methods can respond to these. Addressing these sensory ecological problems will not only help predict how epidemiological events will unfold in a context of globally shifting living environments but also which species might be able to invade a given part of the planet.

Poster Session 2 | Poster Wall 65 | Label: PS2.065

Category: Motor systems, sensorimotor integration, and behavior

Behavioral algorithms of ontogenetic switching in larval and juvenile zebrafish phototaxis

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Animals show major behavioral changes throughout their ontogenetic development. However, the cognitive computations and neural mechanisms controlling this process remain elusive. Here, we use a combination of multiple complementary phototaxis assays and high-throughput behavioral tracking to explore how young zebrafish adjust their brightness preferences while growing from larval to juvenile stage. We observed that larvae are attracted to luminance but repelled by changes in luminance, whereas juveniles are becoming attracted to darkness but remain repelled by luminance changes. Using the observed swim event statistics, we build a library of generative agent-based models, with unique parameter sets for each fish. We validate these models by their predictive power of animal behavior in more complex visual environments. The behavior of both larvae and juveniles can be captured best by a superposition of two competing elements: one element senses the current global luminance level, while the other processes information regarding eye-specific luminance change. We think that the implementation of phototaxis through a competitive arrangement of these two processing streams allows animals to flexibly adapt their behavior in dynamic visual environments, based on their internal state, and their changing behavioral goals during development. Our rigorous model-based dissection approach is a novel way to identify the algorithmic and cognitive changes during ontogeny. To explore the mechanistic implementations of the adjustments in the brain, we will now leverage the rich molecular genetic toolkit and whole-brain single-cell-resolution imaging techniques available for zebrafish, at both stages of development.

Poster Session 2 | Poster Wall 66 | Label: PS2.066

Category: Motor systems, sensorimotor integration, and behavior

Evolution of neural circuits underlying behavioral variation in Drosophila courtship

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From ostrich dances to bowerbird constructions, numerous species have evolved intricate displays to attract mates. The courtship ritual of Drosophila is a powerful system to investigate the evolution of such behaviors. While males of different Drosophila species perform stereotyped displays of distinct motor programs, e.g., chasing or extending one wing to produce song, closely related members of the genus show striking variation in the patterning of these shared motifs and in the elaboration of novel ones. For example, D. melanogaster males chase females closely from behind, while D. yakuba males chase and sing from afar. In contrast to both, D. elegans flaunt their wings in a visual display in front of the female. We examine how visuomotor circuits evolve to drive these variations by leveraging known D. melanogaster courtship circuitry, where prior work suggests male-specific P1 neurons dynamically gate LC10a visual projection neurons, propagating visual information to motor circuits for chasing and wing displays only when males are aroused. To gain insight into the diversification of courtship displays, we have been translating D. melanogaster neurogenetic tools to related species, and using a virtual reality system that allows precise visual stimulus control during simultaneous neural imaging and courtship behavior. We find that across species, activation of P1 neurons drives persistent pursuit and wing extensions towards the same virtual female, indicating their conserved role in modulating the gain of visuomotor pathways. Yet aroused males perform species-specific displays, suggesting visual pathways have evolved to underlie variation in shared motor motifs and to facilitate the emergence of novel motifs. With behavior, optogenetics, and neuroimaging in courting males, we explore how dynamic sensory signals and persistent arousal jointly shape motor sequences to understand how visuomotor circuits evolve to give rise to species-specific behavioral repertoires.

Poster Session 2 | Poster Wall 67 | Label: PS2.067

Category: Motor systems, sensorimotor integration, and behavior

Calcium imaging of neural activity during escape behavior in free-swimming zebrafish

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Studying the neural dynamics that cause adaptive motor responses to variable sensory cues improves our grasp of circuit design. Zebrafish have two startle responses to escape a perceived threat – the short-latency C-start (SLC) and the long-latency C-start (LLC). The SLC is optimized for maximal action in minimal time, while the LLC is optimized for a balance of maximal distance from threat, minimal energy expenditure, and minimal time. The more complex LLC circuit, controlled by a cluster of prepontine neurons, is better at responding to less pressing threats with granular control. It has been unclear which activation patterns start an LLC and how they direct its kinematics. Calcium imaging would be an ideal way to resolve this, allowing us to correlate individual neuron activation and timing with escape selection and trajectory. However, zebrafish rarely produce LLCs when head-immobilized for such imaging. To examine this circuit, we have built a 'swimscope': a microscope that images neural activity in free-swimming zebrafish larvae. The swimscope images a z-stack during the pause after behavior. Using probabilistic maps based on past behaviors, we predict a behavior's likely origin given the imaging area being the endpoint. Once the larva is there, we stimulate an escape with an acoustic stimulus while imaging behavior using a high-speed camera. After completing the escape, the larva pauses. During this period, we interleave imaging a brain slice with moving to the next slice using a high-speed actuator. This images a cellular-resolution whole brain volume using widefield imaging in under a second. We also image unstimulated and immobilized brains as references. We have validated this approach by confirming increased activity in the dorsal raphe of free-swimming y294-Gal4, UAS:GCaMP7s larva after being introduced to the environment. We are now using the swimscope to examine circuits which are intractable to traditional calcium imaging, notably the LLC circuit.

Poster Session 2 | Poster Wall 68 | Label: PS2.068

Category: Motor systems, sensorimotor integration, and behavior

How to be precise – ion channels contributing to spiking precision in vocal motoneurons

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Vocal communication relies on the generation of sounds of different amplitude, frequency and length to meaningfully transmit information. For the generation of such sounds, vocal motor systems rely on precise temporal activation of muscles. This imposes several constraints on the motoneurons (MNs) controlling vocal behaviors, one being precise firing.

One system in which such constraints can be studied is the vocal motor system of synodontid catfish. Synodontids use their elastic spring mechanism to produce sounds through high frequency contraction of muscles coupled to their swim bladder. Muscles contract repetitively in a simultaneous fashion to generate sound pulse series, with individual pulses showing high levels of synchrony. The MNs are adapted to generate these high frequency muscle contractions, as they are endowed with phasic firing properties.

Using patch clamp recordings, we investigated the effect of blocking different potassium channels that might contribute to the phasic and highly precise firing. Application of current steps to MNs triggered firing of action potentials (APs) that occurred with high precision at the onset of stimulation. Recordings at which stimulation did not trigger APs revealed that these firing characteristics were mediated by a rectifying component that occurred shortly after stimulus onset. Preliminary analysis did not reveal significant effects on this component and on MN firing precision after pharmacologically blocking KV-7 and KV-2 channels. Blocking KV-1 channels significantly reduced the rectifying component and onset firing of MNs, thus showing that KV-1 currents play an important role in mediating the highly phasic firing. However, the KV-1 block did not fully abolish onset firing, indicating that other ion channels contribute to this physiological feature of the MNs. In summary, our experiments reveal that a cocktail of different potassium channels mediates precise onset firing in vocal MNs.

Poster Session 2 | Poster Wall 69 | Label: PS2.069

Category: Motor systems, sensorimotor integration, and behavior

Development of vocal responses to social calls in juvenile male zebra finches

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Vocal interactions require the production of reliably timed responses to salient audible signals. Adult zebra finches coordinate their calls with precise response latencies during social interactions with conspecifics. Our study examines how auditory-evoked vocal responses change throughout development to enable coordinated vocal interactions in zebra finches. We tracked call responses of individual juvenile males housed with an adult female companion from 30 until 90 days post hatch (the period of developmental song learning). The juveniles received daily exposure to isochronous call playbacks and their vocalizations were recorded, while simultaneously undergoing song training. We quantified vocal response latencies, rates, temporal precision, and acoustic overlap with playbacks. We found that, on average, response latencies in juvenile birds raised with a social companion were similar to those previously described in adults, and were stable across development. We also observed inter-individual differences in responses: For example, some birds increased their response rates to playbacks across development, whereas others decreased. These findings suggest that the fundamental ability to produce reliably timed auditory-evoked vocal responses is already present early in the developmental period. The capacity for precise interactive call timing could have been acquired through social exposure to calls from the female companion and/or exposure to the isochronous call playbacks. To test if call responses are influenced by developmental social experience, we housed juveniles in social isolation and exposed to call playbacks daily. Additionally, to determine how early exposure to isochronous playbacks affects call responses, we exposed socially isolated birds to playbacks only starting 60 dph. Our work will tease apart the contributions of early social and auditory experience in both the development and maintenance of zebra finch vocal coordination abilities.

Poster Session 2 | Poster Wall 70 | Label: PS2.070

Category: Motor systems, sensorimotor integration, and behavior

Fish couple forecasting with feedback control to chase and capture moving prey

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Predator-prey interactions are fundamental to ecological and evolutionary dynamics. Yet predicting the outcome of such interactions – whether predators intercept prey or fail to do so – remains a challenge. An emerging hypothesis holds that interception trajectories of diverse predator species can be described by simple feedback control laws that map sensory inputs to motor outputs. This form of feedback control is widely used in engineered systems but suffers from degraded performance in the presence of processing delays such as those found in biological brains. We tested whether feedback control could explain predator pursuit maneuvers using a novel experimental system to present hunting fish with virtual targets that maneuvered in ways that push the limits of purely reactive feedback control. We found that predator behavior cannot be explained by purely reactive feedback control, but is instead consistent with a robust pursuit algorithm that combines short-term forecasting of self-motion and prey motion with feedback control. This model predicts both predator interception trajectories and whether predators capture or fail to capture prey on a trial-by-trial basis. Unlike previously proposed models of interception behavior, this strategy need not be precisely tuned to the kinematics of target motion, yet it retains much of the simplicity of pure feedback control, contributing to a generalizable and tractable theory of predator-prey interactions.

Poster Session 2 | Poster Wall 71 | Label: PS2.071

Category: Motor systems, sensorimotor integration, and behavior

A novel conductance-based model of plateau generation

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Plateau potentials are an important but incompletely explored mode of electrical activity in excitable cells, which produce a bout of depolarization that persists long after the end of the precipitating stimulus. This bistability allows the membrane potential to function analogously to an electrical flip-flop, allowing the membrane to stably occupy distinct electrical states. Plateaus serve many physiological functions, from short term state memory to the production of rhythmic movements, and are also implicated in pathologies associated with chronic injuries. It is therefore important to understand the full range of mechanisms that generate plateau potentials as well as their functional significance. We present a new model of a plateau system based on pharyngeal pumping in C. elegans. The model includes a leak current, a synaptic current, a T-type Ca2+ current, a slowly-inactivating L-type Ca2+ current, and a novel K+ current exhibiting ultrafast inactivation. We performed sensitivity analysis of this model to varying sets of maximal conductances. This analysis reveals that the plateau duration is robustly set by the balance between the L-type conductance and the K+ current, allowing in principle for plateaus of arbitrary duration. Furthermore, unlike the canonical delayed-rectifier K+ conductance, the unusual electrophysiology of the K+ channel presented here allows it to activate independently of the plateau duration, delivering extremely rapid repolarization, a crucial feature for the feeding strategy of C. elegans.

Poster Session 2 | Poster Wall 72 | Label: PS2.072

Category: Motor systems, sensorimotor integration, and behavior

Vocal partner familiarity influences call responses and neural activity in zebra finch premotor nucleus HVC

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Zebra finches engage in vocal turn-taking as they exchange affiliative contact calls and they often respond preferentially to specific individuals. To regulate vocal production with respect to the calls of different social partners, the vocal motor pathway must integrate information about relevant acoustic signals. The cortical premotor nucleus HVC has been associated with the context-dependent control of vocal timing during interactions. HVC inhibitory interneurons in particular exhibit auditory-evoked activity patterns in response to calls. However, it is unclear if HVC neuron activity is selectively modulated by calls of specific birds. To test this, we first examined call responses to familiar social partners. We co-housed pairs of birds for a minimum of 5 days, and measured the call response probabilities of males presented with calls from their familiar cage mate and unfamiliar conspecifics. We found that zebra finches preferentially respond to cage mate call playbacks compared to other unfamiliar male or female conspecific calls. To test whether there is a corresponding differentiation of call-evoked activity in HVC, we conducted extracellular recordings with Neuropixels probes in awake male zebra finches while presenting the familiar and unfamiliar call playbacks. We found that approximately half of HVC interneurons showed deviations from their baseline firing rates in response to both familiar cage mate and unfamiliar call playbacks. Nearly one third of interneurons, however, exhibited call playback-specific activity, of which more than 90% were selective for cage mate calls. When comparing the time courses of neural responses for all interneurons with call-evoked activity, we found longer-duration activity patterns were elicited by cage mate calls. Together, these findings suggest a mechanism by which caller-specific and caller-invariant neural representations can influence premotor activity to enable context-dependent vocal responses during interactions.

Poster Session 2 | Poster Wall 73 | Label: PS2.073

Category: Motor systems, sensorimotor integration, and behavior

Tardigaits: coordination and neuromodulation of tardigrade locomotion

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Animals move deliberately through their environment, and much of their nervous system, physiology, and body plan are dedicated to this activity. Tardigrades are 3-mm-long invertebrates that use eight, short, clawed legs for locomotion. They are phylogenetically distant from other animals that move on eight legs, such as spiders and ticks, and are the only members of their ancient phylum (Tardigrada). We recorded the locomotive patterns of a semi-terrestrial species of tardigrades while swimming and walking by video microscopy and kinematic analysis. We found that tardigrade locomotion patterns were variable and could not be described adequately by identifying stereotypic gaits, such as trot, hop, or bound, which have been the focus of studies of locomotion of limbed animals. Only a small fraction of tardigrade locomotive behavior is captured by such gaits. Therefore, we adapted a naïve Bayes classifier (like those used for email spam filters) to distinguish walking and swimming behavior. Both species share similar walking and swimming limb patterns. Walking is characterized by lateral alternation of all four pairs of limbs. At the same time, swimming produces front-to-back propagation of leg motion on each side with motionless hind legs stretched backwards.

Using the Bayes classifier, we tested whether, as in other animals, gait selection is mediated by monoaminergic neuromodulators. We found that bath application of serotonin induces swimming, while dopamine induces crawling in tardigrades. Our approach and use of a Bayes classifier can be applied to other limbed animals to discover patterns beyond stereotypic gaits. These insights could inspire stable multi-legged robots and prompt further research on tardigrades to determine the neural basis of their locomotion.

Poster Session 2 | Poster Wall 74 | Label: PS2.074

Category: Motor systems, sensorimotor integration, and behavior

Neuronal Mechanisms of Visuomotor Stability and Control in Butterflies

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Many animals rely on the detection of optic flow to stabilise self-motion. In Diptera, optic flow is analysed by a population of tangential cells with fan-shaped arborizations in the lobula plate, an output neuropile of the optic lobe. Lobula plate tangential cells (LPTCs) integrate local directional motion signals with receptive fields tuned to detect specific patterns of rotational and/or translational optic flow, a design principle known as 'matched filtering'. LPTCs synapse with wide-field sensitive descending neurons (WFDNs), which project to motor centres in the thoracic ganglia. WFDNs are also matched to patterns of optic flow, reflecting their LPTC input. However, only three WFDNs have been functionally characterised in Diptera. Given such a small population size, what factors determine the patterns of optic flow encoded by LPTCs and WFDNs?

In Calliphora, the distribution of LPTC receptive fields suggests these neurons are tuned to sense and control natural modes of motion during flight. In this case, WFDNs are predicted to convey signals to the thoracic ganglion, inducing stabilizing action of the flight motor to dampen potentially unstable periodic modes of motion. Here we characterize the receptive field properties of a population of spiking WFDN subtypes in butterflies, tuned to specific patterns of optic flow. Some of these WFDNs encode optic flow fields most frequently encountered during flight, such as the exaggerated pitch oscillations which butterflies experience during every wing stroke. This suggests that descending neurons may be matched to stabilise the specific natural modes of motion of the individual species, and may therefore reflect a general design principle for how neuronal circuits are organised for sensorimotor control. The functional organisation of WFDNs may also have implications for the organisation of other descending pathways controlling goal-oriented behaviours, such as those for target tracking and navigation.

Poster Session 2 | Poster Wall 75 | Label: PS2.075

Category: Motor systems, sensorimotor integration, and behavior

Voluntary passive movement – do flies play?

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Play-like behaviour is described across the animal kingdom, with a clear emphasis on vertebrate species. Only few studies so far looked at invertebrate play-like behaviour, and these cases were mostly in a social context.

To address this void, we examined exploratory behaviour in the vinegar fly Drosophila melanogaster by presenting single males to an enriched environment with voluntary access to a spinning platform – a carousel. We also analysed the behaviour of blind or proprioceptive impaired flies in this paradigm to determine the respective influence of visual or proprioceptive input. We demonstrate that flies indeed exhibit complex interaction with a moving carousel with repeated and prolonged visits, but also spontaneous avoidance. These interactions were idiosyncratic but conserved across days in individual animals. Interestingly, carousel visits of flies with different alleles of the foraging gene (Rover and sitter) deviated in their carousel preference from wildtype flies in opposite directions.

We argue that self-exposure to centripetal force represents intentional exafferent stimulation, which may provide an efficient way to improve self-perception and hence motor control. Indeed, male flies that had access to a spinning carousel performed significantly better in a competitive courtship assay than control males, providing evidence for an adaptive value of voluntary passive movement play-like behaviour, or intentional exafference.

Poster Session 2 | Poster Wall 76 | Label: PS2.076

Category: Motor systems, sensorimotor integration, and behavior

Revealing the Dynamics of Danionella dracula fights

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During agonistic contests, the sequence of actions taken by an animal may be influenced by a suite of internal motivational variables that evolve over multiple timescales. The dynamic influence of these internal variables may be apparent from behavioral motifs displayed by the animal over the course of the contest. Male Danionella dracula, a miniature, transparent, teleost fish, engage in fights during which they produce pulsatile sounds, extend their hypertrophied lower jaw, and display gross motor behaviors such as lunges and chases. To examine the influence of motivational states on the temporal structure of behavior during D. dracula fights, we implement a deep learning-based tracking system to capture sound production, jaw extension, and postural behaviors during a male-male resident-intruder assay. We then fit a Markovian process to this behavioral time series to investigate the number and occupancy times of latent behavioral states that each opponent traverses. In doing so, we aim to generate testable hypotheses about the presence of and timescales over which internal motivational state signals operate in the teleost brain. Research support from NIH NS128891.

Poster Session 2 | Poster Wall 77 | Label: PS2.077

Category: Motor systems, sensorimotor integration, and behavior

Changes in respiratory pressure at syllable onset are correlated with variable sequencing of Bengalese finch song

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Complex vocal sequences like human speech involve combining a limited set of sounds in many different orders to produce infinite different signals. Like human speech, the song of the Bengalese finch, a songbird, also consists of a limited set of sounds (syllables) produced as part of variable orders. Specifically, Bengalese finch song consists of two kinds of branchpoint syllables, namely (1) convergent syllables (same syllable followed by different syllables) and (2) divergent syllables (same syllable preceded by different syllables). Previous studies have shown that the acoustic properties of a branchpoint syllable are different for the different orders. Here, we asked whether these changes were correlated with changes in respiratory pressure by recording respiratory pressure and song in Bengalese finches during singing (data from 4 of 12 birds with sufficient songs). Peak inspiratory pressure before the branchpoint syllable was different for the different orders in 5 of 12 convergent syllables and 3 of 13 divergent syllables. Peak inspiratory pressure after the branchpoint syllable was different in 5 of 12 convergent transitions and 4 of 13 divergent transitions. Expiratory waveforms during the branchpoint syllable were different for the different orders. To understand the origin of these differences, we calculated correlations between these waveforms across different orders in 3 different ways, (1) all waveforms aligned to onset, (2) all waveforms aligned to offset and, (3) all waveforms time-warped to the median duration of the syllable. Correlations were largest for offset aligned waveforms. We are currently recording motor activity during singing to understand the neural correlates of these respiratory changes. Overall, our results show the presence of respiratory pressure differences before and during the beginning of such syllables and are consistent with computational models that suggest neural competition at the start of the branchpoint syllable.

Poster Session 2 | Poster Wall 78 | Label: PS2.078

Category: Motor systems, sensorimotor integration, and behavior

A comparative study of temperature resilience in pattern generating circuits

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Temperature fluctuations pose significant challenges to the nervous system of poikilothermic animals that lack active temperature regulation. Decapod crustaceans, key species with massive impacts on food webs in marine coastal ecosystems, are particularly affected by rising habitat temperatures and the dramatic increase in heatwaves that are caused by global warming. A continuous functioning of their nervous system in face of these temperature challenges is critical because it controls vital functions like heartbeat, respiration, digestion, and sensory abilities for habitat selection. Detailed studies from a single crab genus indeed indicate a broad temperature-resilience of the adult nervous system that is enabled through cellular-level matching of ion channel temperature responses and neuropeptide modulation of temperature-sensitive circuits. However, how temperature robustness and acclimatization ability differ between species is poorly understood.

Using the pyloric and gastric mill central pattern generating circuits of the stomatogastric nervous system, we investigate temperature resilience in several decapod crustacean species (Cancer borealis, Cancer magister, Carcinus maenas, Hemigrapsus sanguineus, Callinectes sapidus). Consistent with differences in habitat temperature and exposure to temperature fluctuations, we find that the activity of the pattern generators shows species-specific differences in temperature resilience. Moreover, acclimating animals to different habitat temperature shifted the temperature range in which the pattern generators continued to function. Our results thus demonstrate that neuronal temperature resilience correlates with, and responds to, environmental temperature conditions, enabling us to study the mechanisms underlying temperature resilience.

Poster Session 2 | Poster Wall 79 | Label: PS2.079

Category: Motor systems, sensorimotor integration, and behavior

Deciphering the neural pathway responsible for the initiation and progression of introductory notes in female-directed song of the zebra finch

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Song motif of the male zebra finch, which is a part of its courtship ritual, is a well-studied example of a naturally learned motor sequence. These motifs are repeated multiple times as part of a song bout. Bouts begin with the repetition of an identical, short vocalization called an introductory note (IN). As INs repeat, the intervals between successive INs reduce, and their acoustic properties change to reach a consistent state before the song motif is initiated. Previous lesion studies have shown that the song motor pathway (consisting of motor nuclei HVC and RA) and a dopaminergic nucleus, A11, are important for initiating female-directed songs. However, how these brain nuclei contribute to the initiation and progression of INs remains poorly understood. To address this, we adapted a controlled experimental paradigm (a smart film was used to control the female presentation time) where we could quantify the time taken to initiate INs and the motif after the presentation of a female. We found that birds reliably initiated INs and songs on female presentation (92.92 ± 1.22% of trials with song). The first IN was initiated at 915.29 ± 75.85ms, and the first song motif was initiated at 5467.35 ± 1142.10ms after the female presentation. To examine the contribution of HVC, we carried out HVC lesions (n=2). As shown by earlier studies, following HVC lesions birds produced strings of IN-like repeats (sometimes followed by unknown vocalization). Our preliminary analysis showed that the time to start the first IN and the number of INs remained similar post-lesion, and for 1 bird the intervals between successive INs were longer post-lesion. Taken together with similar qualitative results seen after UVa lesions in other studies, our preliminary results suggest that INs could be initiated by a pathway independent of the song motor pathway while the song motor pathway could be controlling the progression of INs to the song by speeding up the interval between INs.

Poster Session 2 | Poster Wall 80 | Label: PS2.080

Category: Motor systems, sensorimotor integration, and behavior

Characterization of song development in Bengalese finches and the influence of passive tutoring on song

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Bengalese finches are songbirds that sing a limited number of syllables with variable sequencing. They learn their songs as juveniles by imitating a tutor, usually their father. We know that Bengalese finches copy the acoustic structure of syllable types and pairwise transition probabilities between syllables from the tutor and that they can include elements from multiple tutors.

We were interested in characterizing the song development process in Bengalese finches under a live and playback tutoring paradigm. For live tutoring, birds were co-housed in their family group with an adult male and their clutch mates. For playback tutoring, we used a Raspberry Pi-based passive tutoring system with playbacks of songs that contained the song of the father/live tutor, as well as artificially merged song stimuli with elements of the live tutor and elements of other songs. Tutor stimuli were presented to juveniles until day 60 post-hatch in their home cage.

Previous studies have documented the learning process of Bengalese finches either by comparing syllable structure similarity at certain developmental stages or by quantifying sequence transitions once syllable identity is known. Here, we track the emergence of clusters of adult syllable types throughout development (days 30-156) using machine learning and dimensionality reduction techniques and compare them to the emergence of sequence transitions. After the development of the final adult syllable types in the juveniles, we additionally use established methods like syllable duration and tutor similarity to quantify the degree of imitation of the stimuli, and the degree of innovation compared to sibling control clutches that did not receive this tutoring paradigm. Our project leverages recent advances in computational song annotation techniques to gain an overarching perspective on the learning process in this species while being agnostic to arbitrary structures that are difficult to determine in early development.

Poster Session 2 | Poster Wall 81 | Label: PS2.081

Category: Motor systems, sensorimotor integration, and behavior

Axial kinematics and muscle activity during walking and swimming of the centipede Scolopendra subspinipes

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Certain species of centipedes exhibit amphibious locomotion using axial undulation. They walk on land by propagating the leg stepping and axial undulatory waves anterior-posteriorly. These waves shape a coordinated pattern and the wavelength and amplitude of the axial undulation increase as the locomotion speed increases. In water, they swim using axial undulatory waves while the legs are folded along the side of the trunk. Thus, clarifying the control mechanisms underlying the trunk and limb coordination in amphibious locomotion of centipedes would lead to understanding the adaptive motor control system in response to the different environmental conditions. Anderson et al. (1995) measured the locomotion kinematics and electromyograms (EMGs) from the lateral flexor muscles while walking at various speeds. They suggested that the axial undulation during walking is generated not by passive trunk deformation but by trunk muscle activities. However, it remains unclear how the trunk and leg coordination patterns are established via motor control systems and whether the trunk muscle activities during swimming differ from those during walking. Meanwhile, previous studies using mathematical models suggested that trunk-limb coordination can be realized by bidirectional local sensory feedback mechanisms between the trunk and limb as well as the intrinsic neuronal rhythm generation networks (i.e., central pattern generators). To test this hypothesis, as a first step, we aimed to investigate the differences between the axial undulatory waves during walking and swimming regarding both kinematics and EMGs of trunk muscles. Specifically, we used Scolopendra subspinipes and measured the EMGs of left and right trunk muscles at one body segment in the middle during walking (including fast to slow gait transitions) and swimming in water.

Poster Session 2 | Poster Wall 82 | Label: PS2.082

Category: Motor systems, sensorimotor integration, and behavior

Transformation of premotor neural activity and respiratory pressure during the repetition of introductory notes in the male zebra finch

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The song of adult male zebra finches consists of a stereotyped sequence of sounds interleaved by silent gaps and is controlled by the sequential and precise bursting of premotor neurons. This bursting activates vocal and respiratory muscles ensuring perfect coordination between respiration and vocalization during song production. How such sequences are initiated remains poorly understood. Song bouts begin with the repetition of a variable number of short sounds, called introductory notes (INs), before actual song production. Compared to song syllables, INs have more variable gaps between them and more variable acoustic properties. This variability reduces from the first to the last IN suggesting the possibility that INs reflect a “preparatory” phase that drives respiratory and vocal coordination. To test this hypothesis, we characterized IN-related neural activity in two nuclei of the song motor pathway, HVC and RA, in awake, singing zebra finches. In premotor nucleus HVC, individual neurons (n=25; 2 birds) showed complex IN-related activity patterns. Some neurons were active only before the last IN, while others were active for combinations of INs. As a population, this data suggests the presence of sequential bursting during INs with different overlapping subsets of HVC neurons active for each of the INs. In downstream motor nucleus RA, multi-unit activity (n=15 sites; 3 birds) was equal for each IN, while preliminary single-unit activity (n=3 sites; 1 bird) was more variable during IN production. Finally, in a separate set of birds (n=7), we recorded respiratory pressure changes during IN production. We characterized expiratory pressure, inspiratory pressure and, the coordination between respiration and vocalization and all of these parameters were lower during INs as compared to during song. Overall, our results suggest a reconfiguration of the HVC network during INs and increased respiratory-vocal coordination during the last IN, just before song onset.

Poster Session 2 | Poster Wall 83 | Label: PS2.083

Category: Motor systems, sensorimotor integration, and behavior

Development of locomotor behaviour during metamorphosis in the Western Clawed Frog, Xenopus tropicalis

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During frog metamorphosis, locomotion mediated by bilaterally alternating tail swimming is superseded by coordinated movements of newly formed limbs. This transformation provides a window of opportunity for studying the ontogeny of locomotor behaviour. We developed a multi-camera filming arena to record the three-dimensional spontaneous movements of freely swimming tadpoles and to capture changes in swimming behaviour at various stages of metamorphic development. We compare bout frequencies, bout duration, translational and angular speeds, and trajectory shapes to quantitatively describe metamorphic changes in locomotor behaviour. Additionally, we tailored open source 3D pose estimation toolkits (Deeplabcut and Anipose) to reconstruct 3D pose time series of the limbs and tail in freely swimming frogs. Based on the metamorphic changes in inter-limb joint angle coordination, the coupling of tail kinematics with limb joint angles, and correlations of tail and limb kinematics with the 3D trajectories, we hypothesize about circuit-level changes in the organization of spinal locomotor circuits during metamorphosis.

Poster Session 2 | Poster Wall 84 | Label: PS2.084

Category: Motor systems, sensorimotor integration, and behavior

Predictive coding and oscillations underlie the optomotor response in ants

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The optomotor response (OMR) is a spontaneous orientation response to wide-field visual motion. It is assumed to be an innate Stimulus-Response type of behaviour, which predictability is exploited to study various aspects of animals' visuo-motor system. Here we show that the OMR results instead from non-trivial, interacting mechanisms, yielding complex, non-predictable, dynamics such as turning in the 'wrong' direction. We recorded the response of ants tethered within a rotating visual scenery in both open-loop and closed-loop (i.e., when the visual consequence of the ant's turns is added to the external rotation) conditions. On average, ants turned in the direction of the external rotation, as expected. However, closer inspection revealed regular oscillations whose frequency shifted with the visual-motion perceived; showing that the OMR implies the modulation of an intrinsic oscillator. Furthermore, ants in open-loop condition displayed more chaotic responses than in closed-loop. Critically, in open-loop we even saw important reversals in turning direction occurring randomly over time. This shows that the OMR is not mediated by the 'detected' visual-motion signal but via the computation of 'prediction-errors', a non-trivial cognitive feat. Finally it appears that at least in ants, the OMR is not explained by a feedforward Stimulus-Response like mechanism, but by the computation of prediction-errors playing with an endogenous oscillator.

Poster Session 2 | Poster Wall 85 | Label: PS2.085

Category: Motor systems, sensorimotor integration, and behavior

Hopper by name, hopper by nature: Investigating the locust startle response using whole-brain functional imaging

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Swarms of the migratory desert locust Schistocerca gregaria can extend over several hundred square kilometers, devouring crops and vegetation. Despite their immense socio-economic impact, the mechanisms, underlying their collective decision-making processes ultimately determining their movement patterns, still need to be better understood. Therefore, we combined behavioral experiments of freely walking animals with fine-scaled extracellular recordings of descending neurons and functional whole-brain calcium imaging to shed light on these vital processes.

Navigating complex environments while avoiding threats is central to survival. In order to make an informed escape decision, animals rely on individually acquired information, such as an approaching looming silhouette of a predator. Evaluating these effectively is crucial for an informed escape decision, that minimizes false positive errors, consequently conserving valuable energy. In our study, we investigate the initiation of startle responses in gregarious desert locusts to unravel their underlying decision-making processes. Controlled lab experiments with single, freely moving animals provide insights into the characteristics influencing individual startle response, revealing a non-linear relationship between stimulus intensity and response probability. We characterize neuronal evidence accumulation processes by combining these stimulus-response characteristics with recordings from descending motion detection neurons. Moreover, we are now establishing a protocol for whole-brain functional imaging to further broaden the understanding of involved brain regions with single-neuron resolution. Taken together, we aim to shine a new light on the principles underlying the locust escape decisions by employing an overarching, naturalistic approach to investigate the collective decision-making processes of a devastating pest species.

Poster Session 2 | Poster Wall 86 | Label: PS2.086

Category: Motor systems, sensorimotor integration, and behavior

Patterns of inhibition in fly grooming behavior reveal neural circuit organization

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Patterns in fly grooming behavior suggest how neural circuits in its brain and ventral nerve cord organize this action sequence. Drosophila groom themselves with periodic leg movements directed toward different body parts. Leg sweeps and rubs alternate with characteristic frequencies, and there is a gradual progression from anterior to posterior as dust is removed. Our previous large-scale behavior quantification showed rhythmicity over distinct time-scales, from individual leg sweeps (~7Hz) to alternating bouts of head sweeps and leg rubs (~0.5Hz). We compared grooming in Drosophila species and assessed individual variability to show stochastic progression is a conserved feature. Using genetic/neuronal screens and connectome analyses, we identified sensory, motor, and command neurons that control individual cleaning sub-routines. We now use this understanding of grooming behavior and tools to control activity in specific neurons to map circuits that coordinate movement of multiple joints within a leg, contribute to rhythmicity, govern different phase relationships between legs, and trigger transitions between them.

Higher spatio-temporal resolution video tracking with DeepLabCut shows which combinations of leg segments move together, reflecting coordinated control by both excitatory and inhibitory pre-motor circuits. Optogenetic perturbations of inhibitory neurons reveals an unexpected degree of contribution to movement timing. Anatomical evidence shows both sensory feedback and motor re-afferents to the leg pre-motor circuits: perturbation experiments to test functions are underway. Behavioral characterization and connectome mapping indicate that neural control of fly grooming is accomplished by circuits that coordinate different groups of muscles to work together in synergies and that the ability to switch between synergies provides flexibility to produce the range of cleaning movements the fly requires.

Poster Session 2 | Poster Wall 87 | Label: PS2.087

Category: Motor systems, sensorimotor integration, and behavior

Alarm pheromone modulates directional decision-making in the Mauthner-cell startle circuit

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Successfully navigating a threatening encounter requires appropriate decision-making, such as escaping away from an approaching predator. However, a predator encounter also induces acute stress in prey, which putatively can disrupt optimal decision-making leading to maladaptive behavioral responses. Here, we tested the effect of an alarm pheromone (i.e. a natural stressor) on Mauthner cell (M-cell) evoked startle escapes (C-starts) in response to directional acoustic stimuli. Typically, the M-cell closer to the stimulus source fires first, while simultaneously inhibiting the contralateral M-cell, which leads to a C-start away from a threat. Individual goldfish (N=5, per group) were either exposed to saline or the pheromone. The results showed appropriate away startle directionality in the saline group but not in the pheromone one (Saline: 67%; away; p=0.003; Pheromone: 59%; away; p=0.114; one-sided binomial test with Ha: Prob. >0.5). Consistently, we found goldfish (N=6, per group) injected with either saline or cortisol (IP; 0.5 mg/g) also showed a disruption of directionality (Saline: 73%; away; p=0.004; Cortisol: 68%; away; p=0.054; one-sided binomial test with Ha: Prob. >0.5). Interestingly, when we tested groups of two goldfish (N groups=7), in a within experimental paradigm, we did not observe a disruption of directionality with the pheromone (Baseline condition: 69%; away; p=0.038; Pheromone condition: 81%; away; p=0.004; one-sided binomial test with Ha: Prob. >0.5). One possible explanation for the latter result might be that a companion effect/social buffering dampens the stress effects of the pheromone. Taken together, the results suggest that an acute stressor affects the decision-making process within the M-cell circuit. More broadly, stress might bias the cost-benefit analysis of a decision toward an apparent suboptimal response by introducing more uncertainty/unpredictability.

Poster Session 1 | Poster Wall 88 | Label: PS1.088

Category: Metabolism, biological rhythms and homeostasis

Electrophysiological Characterization and Computational Modeling of Insulin-Producing Cells in Drosophila

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Insulin-Producing Cells (IPCs) are modulatory neurons within the insect brain, which release Insulin-like Peptides (ILPs) and exert profound influence across various facets of the organism's physiology. ILPs play a central role in metabolic regulation and the modulation of behavior, making them an intriguing subject of study. Drosophila melanogaster is an ideal model organism for investigating these neurons on a fine-grained scale, due to a wide availability of genetic tools, well-established experimental techniques and the near complete mapping of brain connectivity. However, the study of IPC activity and insulin release remains challenging: IPCs are interconnected with a complex network of modulatory neurons that have a wide variety of effects on IPCs and form numerous direct, indirect, and often reciprocal connections. Due to the complexity of this system, a pure experimental approach is insufficient to develop a comprehensive understanding of IPC population dynamics. Theoretical models are powerful tools to study the intricacies of such interactions in a coherent framework. Hence, we decided to use a combined approach of electrophysiological characterization and computational modeling to study IPCs and the complex modulatory network they are part of. Here, we developed a conductance-based, Hodgkin-Huxley model of IPCs using data from patch-clamp recordings and optogenetic functional connectivity experiments, in order to quantify the intrinsic electrophysiological properties and to characterize some of the key modulatory inputs of this neuronal population. Optimization of our model, performed over a large number of parameters to fit our experimental data, will develop a high-fidelity reproduction of the IPC network. This data-driven model will allow us to analyze how circuit-level changes with different temporal dynamics produce the global population activity of IPCs, contributing to a better understanding of how neuromodulation shapes metabolism and behavior.

Poster Session 2 | Poster Wall 88 | Label: PS2.088

Category: Motor systems, sensorimotor integration, and behavior

Representations of floral features for foraging tasks in the neck connective of the tobacco hawkmoth, Manduca sexta

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The descending connective of invertebrates is often hypothesized to carry task-level representations of essential visual features tuned to an animal's behavior and ecology. Target selective descending neurons (TSDNs) enable pursuit in several phylogenetically disparate insects. TSDNs are selective for object size of ~5 degrees and lack of wide-field motion responsiveness. However, other insects have different tasks. Many agile hawkmoths use vision and other cues to localize and hover-feed from flowers during foraging. The hawkmoth connective contains putative object-sensitive descending neurons (OSDNs) that are broadly directionally responsive but with unknown size tuning. Target selectivity could be widely conserved even in non-pursuit species or OSDNs could be tuned for moth behavior. To test this, we collected 67 visually responsive units using a new neck connective multielectrode array preparation. Using unsupervised clustering we identified three clusters that were responsive either to narrow bars or to wider flower-like stimuli. Receptive fields were predominantly unilateral but broad and close to the dorsoventral midline. We found no neurons tuned as small target detectors. Instead, we found one class that responded to both intermediate-size bars and flowers, but tuned to 20 and 30° in height, consistent with flower sizes during foraging. However, these units are unlikely homologous to TSDNs because they are also wide-field motion responsive. Another cluster lacked motion responsiveness and did not respond to flower-shaped stimuli, but were selective for narrow bars. These units could enable tracking of narrow visual nectar guides used during proboscis placement and may represent modified TSDNs. Nonetheless, spike-rate encoded features are likely too slow to fully describe the behavioral dynamics Taken together our results suggest that some DNs in the hawkmoth are tuned not for small, prey-like targets, but for features necessary for their feeding behavior.

Poster Session 2 | Poster Wall 89 | Label: PS2.089

Category: Motor systems, sensorimotor integration, and behavior

The AMsh glia of C. elegans modulates the duration of touch-induced escape responses

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Once considered mere structural support cells in the nervous system, glia have recently been demonstrated to play pivotal roles in sensorimotor processing and to directly respond to sensory stimuli. However, their response properties and contributions to sensory-induced behaviors remain little understood. In Caenorhabditis elegans, the amphid sheath glia (AMsh) directly respond to aversive odorants and mechanical stimuli, but their precise transduction machinery and their behavioral relevance remain unclear.

We investigated the role of AMsh in mechanosensation and their impact on escape behaviors in C. elegans. We found that nose touch stimuli in immobilized animals induced a slow calcium wave in AMsh, which coincided with the termination of escape reversal behaviors. Genetic ablation of AMsh resulted in prolonged reversal durations in response to nose touch, but not to harsh anterior touch, highlighting the specificity of AMsh's role in distinct escape behaviors.

Mechanotransduction in AMsh requires the α-tubulin MEC-12 and the ion channels ITR-1 and OSM-9, indicating a unique mechanosensory pathway that is distinct from the neighboring ASH neurons. We find that GABAergic signaling mediated by the GABA-A receptor orthologs LGC-37/8 and UNC-49 play a crucial role in modulating the duration of nose touch-induced reversals.

We conclude that in addition to aversive odorant detection, AMsh mediate mechanosensation, play a pivotal role in terminating escape responses to nose touch, and provide a mechanism to maintain high sensitivity to polymodal sensory stimuli.

Poster Session 2 | Poster Wall 90 | Label: PS2.090

Category: Motor systems, sensorimotor integration, and behavior

Functional analysis of the locomotor circuits in the ventral nerve cord of Drosophila melanogaster

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Limb coordination is a complex motor control task requiring the integration of top-down, goal-oriented commands and fast sensory-motor reflex loops. In the fruit fly, Drosophila melanogaster, the ventral nerve cord (VNC) is the locus of the control circuits for the six legs of the insect. However, the circuits and mechanisms of the limb control and coordination in the fruit fly are largely unknown. Informed by the VNC connectome data, we have used the targeted gene-expression system, split-GAL4, to create a set of driver lines that allow us to label and manipulate individual cell types within the VNC. These include intersegmental neurons are likely to be essential to limb coordination. To test the functional role of these neurons, we have performed an inactivation screen on 670 split-GAL4 lines using the light-gated chloride channel, GtARC1, to selectively silence cell types while recording the behavioral consequences. We used our Fly Disco automated analysis pipeline to process the resulting 7095 videos, totaling 461,175 hours of fly behavior. Each video was tracked with FlyTracker for body tracking, registered, and then keypoint-tracked using our Animal Part Tracker (APT) software to accurately detect the pose of each fly (7-body, 10-leg, and 4-wing keypoints). This pose tracking allows us to perform detailed analysis of the locomotor behavior of the flies. From the leg tips tracking data, we can compute a suite of engineer features to quantify the limbs's movement and coordination. We are using these features to identify movement and coordination deficits via machine learning-based analysis. Taken together with the circuit data from the VNC connectomes, we are characterizing the functional role of VNC neurons and circuits in limb control and coordination.

Poster Session 2 | Poster Wall 91 | Label: PS2.091

Category: Motor systems, sensorimotor integration, and behavior

The Mauthner array is necessary to generate distinct types of escapes in zebrafish larvae

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Segmentation in the hindbrain plays an important role in the organization of motor circuits in vertebrates and gives rise to sets of homologous neurons that share similar morphology and functionality. A key question in the field is what purpose does segmental homology serve? One hypothesis defends that using homologous neurons in different combinations allows for modulation of the kinematics to produce movement diversity. Another possibility is that the homologues are employed independently to create the same type of movement, allowing for redundancy in the motor system. The escape behavior of larval zebrafish is ideal to test these hypotheses. First, larval escapes can be triggered by three sensory modalities (somatosensation, audition and vision), opening the possibility that there are modality-specific types of escapes. Second, escape behaviors are generated by the Mauthner array, a series of 7 pairs of homologous neurons (Mauthner cell, MiD2(cm/cl/i), and MiD3(cm/cl/i)), that are distributed throughout 3 segments and that can be identified from fish to fish. Here, through a series of behavioral experiments, we show that zebrafish larvae produce distinct types of escapes when using different sensory modalities. That is, when using somatosensation or audition, larvae employ short latency C-starts (SLCs) and long latency C-starts (LLCs), that have been previously described. However, when larvae use vision to escape a virtual predator they produce another type of escape, that we called burst SLC because it has high tail-beat-frequency. Furthermore, by performing 2-photon laser ablations of the M-array, we show that the three escape types are abolished, suggesting that the M-array is essential to generate escape diversity. Since all reticulospinal homologues were generated by duplication of ancestral segments our results support the idea that hindbrain segmentation exists to increase movement diversity and allow animals to have a wider repertoire of movements.

Poster Session 2 | Poster Wall 92 | Label: PS2.092

Category: Motor systems, sensorimotor integration, and behavior

MATREX VR: Real Animals Forming Virtual Swarms through the Matrix

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Collective behavior emerges from the spontaneous interplay of individual choices and interactions, orchestrating a spectacle across Earth, Air, and Water that is as captivating as it is complex to decipher. To navigate this complexity, we built MATREX, inspired from ‘Prakruti Maye’, wherein the tangible and the illusory interlace, manifesting an environment that subtly disrupts the boundaries between actuality and artifice. It’s not a replication of an ecosystem but an illusion of one, gently guiding senses through an intricately constructed journey. MATREX stands for MATREX Architecture Terraforming Realistic Environments in X; X∈{Earth,Air,Water}. At its core, the MATREX enables isolated individuals, each positioned within their own arenas, to perceive and interact with each other in real time. Thus, crafting virtual swarms composed of real individuals, behaving as though in the natural world. To achieve this, we generate panoramic naturalistic input using high refresh rate, commercial LED panels and off the shelf parts. We designed parametric, 3D printable modules to accommodate a wide range of organisms from Drosophila to a full adult Locust, paving the way for modular, low cost, and scalable arenas for studying walking and flying collectives. Advancing beyond traditional yaw-focused setups, our flight system features a fully 3D printable, 6-axis force-torque sensor capturing pitch, yaw, roll and translational forces. Our open source system simplifies the intricate data capture process and democratizes access to high throughput VR research. With its unique capacity to probe the neural basis of individual and collective decision-making, MATREX VR takes us a step closer to deciphering the complex interplay of individuals in collectives and untangling the enigma it presents.

Poster Session 2 | Poster Wall 93 | Label: PS2.093

Category: Motor systems, sensorimotor integration, and behavior

Is the motor program of faster insects more temporally precise?

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Do faster animals encode information with greater temporal resolution, or do other factors contribute to allowing some animals to locomote at high frequencies? Appendages move in cycles, so muscles have to be orchestrated relative to where the limb is in a cycle. But as locomotion frequency gets faster, if muscles are purely orchestrated relative to the phase of limbs this would require the nervous system to become increasingly more temporally precise. Does the motor program actually become more precise to scale with phase, or do limitations in biophysical timescales of muscles and neurons force different motor control strategies? We sought to test this using Bombycoid moths, a clade of flying insects that share similar neuromuscular anatomy despite different flight styles, ecological niches, and, crucially, wingbeat frequencies. We recorded the comprehensive, spike-resolution motor program for flight in tethered individuals from a variety of species with wingbeat frequencies ranging from 13 to 50 Hz. From this data, we find that within and across species spike count decreases with increasing wingbeat frequency. We also find that coordination patterns of the motor program and spike timing variance scale differently than naive phase scaling would predict, indicating differing strategies for flight muscle coordination as frequency increases. We then show that spike timing precision for all muscles in all species is constant at ~1 ms, indicating that faster species do not get commensurately more temporally precise. This evidence together indicates that absolute timing, more than phase, is preserved as frequency increases. To finish, we show how this observed scaling of the motor program connects to changes and adaptations in muscle and neural physiology. As a complete picture, our work indicates that to locomote at higher frequencies, animals cannot play the same motor program faster; instead, unique frequencies of locomotion require unique neuromechanical strategies.

Poster Session 2 | Poster Wall 94 | Label: PS2.094

Category: Motor systems, sensorimotor integration, and behavior

Phototaxis in the primitive chordate Ciona: orienting behavior and neural circuits

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Tunicates, including Ciona intestinalis, are a diverse group of invertebrate chordates. Ciona start life as free-swimming larvae that disperse in the marine environment. After several days, larvae undergo metamorphosis to form a sessile adult. The conserved anatomy of Ciona with vertebrates is most evident at the larval stage. Ciona larvae have notochords running the length of their muscular tails, and central nervous systems with homologs of the vertebrate forebrain, midbrain, hindbrain and spinal cord. However, the Ciona larval CNS contains only ~180 neurons, which has facilitated the full description of its connectome. Ciona larvae display a number of behaviors, including a looming-shadow startle response, negative gravitaxis and phototaxis, and a mechanosensory escape response. The connectome has allowed for identification of core sensorimotor circuits of all of these behaviors. The core negative phototaxis circuit consists of twenty-three ciliary-type photoreceptors. The photoreceptors project directly to a group of nine neurons in the midbrain called photoreceptor relay neurons (prRNs). The prRNs then project to the hindbrain where they synapse to a group of six secondary interneurons called motor ganglion interneurons (MGINs). Finally, the MGINs synapse to motor neurons in the hindbrain and spinal cord. Negative phototaxis in Ciona consists of two distinct and separable behaviors. First, the larvae perform short spontaneous casting swims. If the direction of light is detected during casting, a sustained negative phototaxis swim is initiated. Our research has shown that casting is a rhythmic behavior with a period averaging ~15 seconds, but with extensive variation in the period. The prRNs are cholinergic and appear to have intrinsic activity that is under tonic inhibition. Our most recent findings point to a group of hypothalamic neurons that project to the prRNs, and which show weakly-coupled oscillations with periods of ~15 seconds, as drivers of casting.

Poster Session 2 | Poster Wall 95 | Label: PS2.095

Category: Motor systems, sensorimotor integration, and behavior

Dopamine guides vocal learning through reinforcement

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Many of our motor skills like speaking or playing a musical instrument are acquired through a process of trial-and-error learning. It has long been hypothesized that dopamine plays a critical role in this process, motivated by two main findings. First, dopamine in the basal ganglia is thought to guide reward-based trial-and-error learning by encoding reward prediction error, decreasing after worse-than-predicted reward outcomes and increasing after better-than-predicted ones. Second, by changing the perceived song quality with distorted auditory feedback, our previous work in adult zebra finches showed that dopamine in Area X, the singing-related basal ganglia, encodes performance prediction error; dopamine is suppressed after worse-than-predicted (distorted syllables) and activated after better-than-predicted (undistorted syllables) performance. Here we parametrize developmental song learning trajectories in juvenile zebra finches into acoustic features and use fiber photometry to monitor concurrent dopamine activity in Area X. Dopamine was activated after syllable renditions that were closer to the eventual adult version of the song and suppressed after syllables that were farther away. Fitting the song trajectory to an actor-critic reinforcement learning model showed that dopamine corresponds to the prediction error term. The correlation structure between dopamine and song fluctuations revealed that dopamine activity attended to different song features over development and the direction in song feature space of maximal correlation with dopamine predicted the future movement of song. Reinforcement learning has contributed significantly to the current revolution in artificial intelligence. Our results show that complex natural behavior in biological systems can also be learned through dopamine-mediated reinforcement learning.

Poster Session 2 | Poster Wall 96 | Label: PS2.096

Category: Motor systems, sensorimotor integration, and behavior

Dissecting swim-to-walk transition and its neural basis during Xenopus frog metamorphosis

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Spinal neural circuits comprise molecularly and functionally diverse neurons that directly control the precision, speed, and adaptability of movement. To dissect how molecular cell-type diversity influences motor output, we examine the unique swim-to-walk transition of frog metamorphosis. We combine a robust behavioral assay with molecular cell-type profiling in wild-type, and loss-of-function CRISPR mutant, animals and evaluate how motor and interneuron subtypes scale with, and contributes to, tail versus limb movement.

Using a high-speed behavioral setup, we track tadpole and frog body parts, quantify complex movement, and create locomotor profiles across metamorphosis. We then register this behavioral data with spinal cell-type composition. We profile the expression of transcription factors that define motor neurons and V1 interneurons, an inhibitory class modulating motor neuron firing. In free-swimming tadpoles, both motor and interneuron populations double in number and diversify in their transcriptional profile, acquiring subpopulation diversity including motor neuron columns and V1 clades. With limb emergence, these cell types peak in their number and largely match the molecular diversity of the neonate mouse.

We then use loss-of-function analysis to evaluate the contribution of two emergent spinal neuron subtypes to the tadpole versus frog motor repertoire. Using CRISPR/Cas9, we selectively knocked out two master regulator genes responsible for motor neuron or V1 specification, respectively: FoxP1 and Engrailed-1. By comparing the behavior of mutant versus wild-type animals, we show a differential role of each subtype in tail and limb movement range, speed, and frequency.

Our work maps MN and V1 molecular properties onto motor behavior during frog metamorphosis, defines how transcriptional diversity scales with movement complexity, and demonstrates spinal neuron cell type conservation and divergence across tetrapods.

Poster Session 2 | Poster Wall 97 | Label: PS2.097

Category: Motor systems, sensorimotor integration, and behavior

Neural Circuits Underlying Optomotor Responses in Larval Teleost Fish

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While many sensory cues appear continuously, animals often respond with discrete movements with a defined start and end. For instance, to catch a flying ball, the brain converts continuous visual motion into specific arm movements. Similarly, the optically and genetically accessible larval zebrafish stabilizes its position in response to continuous optic flow by moving in short burst-and-glide swims (i.e., bouts), performing the optomotor response (OMR). Yet, the larval Danionella cerebrum, a closely related translucent teleost, moves in continuous swims. These natural behavioral differences provide a unique opportunity to investigate and compare the neural encoding of this sensorimotor transformation. Here, we characterize larval Danionella OMR behaviors by presenting direction and eye-specific motion patterns in freely-swimming, closed-loop tracking assays. Comparing OMR kinematic metrics, including locomotion direction, velocity, and duration, between larval Danionella and zebrafish, we show that larval Danionella follow the direction of optic flow comparable to zebrafish, but swim continuously at a slower speed. To investigate the natural variation of the underlying neural mechanisms of these visually evoked behaviors, we performed brain-scale, head-fixed, volumetric two-photon calcium imaging with simultaneous high-speed tail tracking. Both species contain a multitude of diverse motion-responsive neurons in homologous, visual processing brain regions, such as the retinorecipient pretectum and anterior hindbrain. We show different temporal dynamics across the mid and hindbrain of both species, particularly encoding visually driven motor behaviors. Our results suggest species-specific visuomotor transformation from sensory to descending motor command centers. This study provides a neuroethological framework to identify the neural mechanisms underlying discrete and continuous locomotion in vertebrate sensorimotor transformations.

Poster Session 2 | Poster Wall 98 | Label: PS2.098

Category: Motor systems, sensorimotor integration, and behavior

Neural mechanisms underlying task-specific activity of pre-motor interneurons and motor neurons of an insect leg

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While walking, movements of the legs are continuously adapted to behavioral and environmental demands, a complex task given the multi-jointed nature of legs. One of the considerable easy cases is changing walking direction, i.e. stepping forward (fw) versus backward (bw). Insect middle legs are well suited for studying the mechanisms contributing as they are the least specialized legs. Both stepping directions differ mainly in the activity pattern of coxal motor neurons (MN) and muscles, i.e. the retractor (Ret) and protractor (Pro) coxae, however, only merely for MNs innervating muscles of the two distal leg joints, i.e. the coxa-trochanter (CTr) and the femur-tibia joints (FTi; Rosenbaum et al. 2010). While in fw stepping Ret MNs are active during stance and Pro MNs during swing, the opposite is true for bw stepping. In our study on the stick insect we focused on the neural control of fw and bw stepping using pharmacology, deafferented as well as semi-intact preparations combined with extra- & intracellular recordings. We started out by analyzing the synaptic drive MNs receive from their respective joint CPGs: rhythmic activity of all recorded MNs is based on rhythmic inhibitory input, except for depressor MNs, which were found to receive primarily phasic excitatory input (Ruthe et al. 2024). Then, we studied the activity of premotor nonspiking interneurons (NSI) in both stepping directions to identify, how NSIs contribute to the generation of forward or backward stepping: so far, NSIs of the CTr and FTi premotor networks show qualitatively similar activity patterns in both situations. In contrast, NSIs of the coxal premotor network fall into two groups: They either reverse their activity pattern or show the same activity pattern along with a change of walking direction. We are currently investigating how these two groups of NSIs contribute to MN activity in both fw and bw walking.

Poster Session 2 | Poster Wall 99 | Label: PS2.099

Category: Motor systems, sensorimotor integration, and behavior

An efference copy suppresses optomotor responses in flying Drosophila

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During voluntary behaviors, animals need to disable any reflexes that could interfere with the intended movements. With the optomotor response, flies stabilize a straight flight path by correcting for unintended deviations sensed as the panoramic motion of the surround. HS cells of the fly are thought to mediate optomotor responses to horizontal motion. During spontaneous flight turns, a putative efference copy acts on HS cells to counteract the visual input elicited by the fly's own behavior. Here, we investigated whether looming-elicited saccades have a similar effect on HS cells, and how this may affect the reafferent motion vision by intracellular recording in behaving flies. Through 3D modelling of the visual input, we demonstrate that insects experience a complex reafferent optic flow during saccadic flight. Surprisingly, the reafferent HS cell response is biphasic, thus reconciling our findings in vivo with the efference copy hypothesis. At the same time, modelling provides evidence for functional subdivision of HS cells. Furthermore, we investigated a descending neuron pathway that offers an outcome for the efference copy signal to impact flight behavior.

Poster Session 2 | Poster Wall 100 | Label: PS2.100

Category: Other

A neural path for visual discrimination of magnitudes in zebrafish

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The ability to deal with quantity developed from an evolutionarily conserved system for approximating non-symbolic numerical magnitude. Neuronal systems selectively tuned to the number of items of a set have been described in the parietal and prefrontal cortex of human and non-human primates and equivalent areas in crows and young chicks. However, little is known about the neural correlates of this ability in fish.

Combining a habituation/dishabituation paradigm with molecular biology assays, we have recently identified part of the neural network associated with quantity discrimination in adult zebrafish brains. Fish were habituated to sets of 3 or 9 red dots for four consecutive days. During dishabituation, zebrafish faced a change (i) in number (from 3 to 9 dots or vice versa), (ii) in shape (3 or 9 red squares), or (iii) in size. A control group was tested with the same stimuli as during the habituation. After thirty minutes, their brains were dissected to quantify immediate early gene expression.

Results showed an involvement of the retina and optic tectum in encoding continuous magnitude. We also found a role of the habenula, the optic tectum, the preglomerular complex, and the caudal regions of the dorsolateral and dorsocentral pallium, in the encoding of discrete magnitude (e.g., change in numerosity).

In conclusion, our results suggest an early involvement of thalamic and tectal areas for encoding of continuous quantity, and of more pallial (via tectal and thalamic nuclei) regions for discrete quantity.

This study may provide the foundation for using zebrafish as an experimental model to explore developmental dyscalculia, a human disability associated with learning and comprehending quantity.

Poster Session 2 | Poster Wall 101 | Label: PS2.101

Category: Other

Custom-tailored viral vectors for protein expression in the quail's brain

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Comparative studies in cognitive neuroscience across different species are of high interest and importance. However, research in non-standard animal species, such as birds, is delayed due to difficulties in applying molecular and genetic tools for imaging and manipulating neurons. Viral delivery stands out as one of the most significant limiting factors in this regard. Indeed, we found that the transduction efficiency of Japanese quails' neurons by various commercially available AAVs yields minimal to complete absence of transduction of neurons in vivo. We, therefore, set out to tailor AAVs for quail neurons. To achieve this goal, we developed a unique protocol for culturing primary neurons from quail embryos for a higher throughput screen of AAVs. AAV1 exhibited some infectivity, albeit poorly, likely stemming from the partial compatibility of AAV1's capsid with the quail's diverging AAV-Receptor. We thereby rationally re-designed the capsid of AAV1 by introducing a single point mutation (AAV1-T593K; AAV1*). AAV1* demonstrated enhanced transduction efficiency in vitro and in vivo. In a parallel thrust, we have also explored a naturally occurring quail AAV (qAAV) and, surprisingly, found that it exhibits enhanced tropism towards the transduction of quail neurons in vitro. We now aim to employ these new variants to explore neural activity in awake and behaving quails and improve their transduction capacity towards quail neurons.

Poster Session 2 | Poster Wall 102 | Label: PS2.102

Category: Other

Molecular basis of parasite-induced behaviors in the golden shiner (Notemigonus crysoleucas)

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Trophically transmitted parasites often manipulate host phenotype (i.e., morphology, physiology, and/or behavior) to increase susceptibility to predation by their definitive hosts. Parasite-induced behavioral changes can be triggered via modification of neuronal functioning, by interfering with the communication between the immune and nervous systems in the host (e.g., cytokine signaling), and/or by manipulating host hormones such as cortisol or neurotransmitters like serotonin. Cyprinid fish parasitized by trematodes of the genus Posthodiplostomum are predated upon more frequently than unparasitized fish, suggesting that Posthodiplostomum sp. infection alters or suppresses antipredator behaviors in host fish. However, the specific pathways have not yet been studied and a wide range of potential physiological changes in host neural, endocrine, and immune systems could be involved. Here, we examined how variation in infection intensity of Posthodiplostomum sp. metacercariae altered neuroendocrine-immune markers in golden shiners (Notemigonus crysoleucas). We measured brain serotonin (neural), interleukin-1ß (IL-1 ß immune), and cortisol (endocrine) to assess correlations with infection intensity. Microdissection and enzyme-linked immunosorbent (ELISA) assays were employed to analyze serotonin in neurochemically relevant brain regions of parasitized fish. Cortisol and IL-1ß levels were measured using ELISAs on whole body homogenates. These results were then used to establish associations with parasite load. It is hypothesized that brain serotonin, IL-1ß, and cortisol levels will be altered in relation to parasite load. Results are discussed in the larger context of the mechanisms parasites may use to alter taxis, sensory processing, mood and cognition of their hosts and how this information can provide neuroscientists with novel insights into natural phenotypic engineering to create a novel phenotype.

Poster Session 2 | Poster Wall 103 | Label: PS2.103

Category: Other

Neuronal layers in the dorsal telencephalon of the peacock gudgeon

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Within the mammalian forebrain, the six-layered isocortex marks a hallmark of vertebrate brain evolution. Such a layered organization facilitates complex parallel and reciprocal processing of information. While most prominent in mammals, cortex-like structures have also been found in birds and non-avian reptiles. The dorsal telencephalon of teleost fish, however, is known for an entirely different form of organization. Here, neurons are arranged in different nuclei or broader forebrain regions. One partial exception to this general organizational principle are gobiid fish. Within their dorsal telencephalon, neurons are arranged into differentiated layers (Dx), consisting of four fiber-dense and 5 soma-packed layers.

Immunohistochemical experiments revealed a differential immunoreactivity of the fiber-dense layers to parvalbumin, substance P and ChAT respectively. In-vitro-tract tracing depicted the preglomerular complex (PG), a thalamus-like multisensory relay station, as the major ascending, extra-telencephalic source of information to Dx. Within the dorsal telencephalon, Dx is highly interconnected with Dc, Vc1, vVd and Vl. Using single-cell DiI-injections and multi-cell ballistic labelling with a gene-gun and DiI-bullets, we found that neurons within Dx show a great morphological variability, of small sized somata, many packed with dendritic spines. Calcium imaging experiments showed that only the activation of a subregion of Dc resulted in activation of Dx. Whether the different layers are recruited in a temporarily ordered fashion, which would indicate parallel processing, remains to be investigated.

Poster Session 2 | Poster Wall 104 | Label: PS2.104

Category: Other

Shared tribulations of neuroethologists and behavioural ecologists

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Neuroethologists often use anatomy to explain behaviour ('species x is smart because it has a complex brain'), while behavioural ecologists predict and/or explain it by its adaptive consequences. Neither can work on its own. For instance, classical optimal foraging theory started by building models that predicted patch exploitation and diet choice in stylised situations, from the premise of maximisation of average long-term rate of returns. Later elaborations considered variance and skew in outcomes (risk), perception processing universalities (Weber's Law), temporal discounting (intertemporal choice), multiple agent situations (game theory) and more, striving to better represent the environments of evolutionary adaptation and to respond to weak predictive performance. Unfortunately, anybody who has seen a hamster allocating time, energy, and effort to its running wheel knows that even with those enrichments, animals' decisions can be hard to predict from either consequences or brain anatomy. Effective predictive models need to rely on a somewhat tautological ploy common in microeconomics, namely inferring utility from observed preferences, predict as yet unobserved behaviour from utility maximisation, and then adjusting hypotheses about adaptive significance while seeking neural correlates. This means that behavioural biologists must oscillate between functional and proximate levels (Tinbergen), must conceptualise decision systems in their ecological context (Simon), and must be aware that a given environment's 'affordances' are agent-dependent (Gibson). This makes ethological research unavoidably interactive (and fun). I will illustrate these theoretical claims with pre-existing and ongoing behavioural findings.

Poster Session 2 | Poster Wall 105 | Label: PS2.105

Category: Other

Mapping the neural basis for individual differences in the exploratory behavior of adult zebrafish

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Individual differences in behavior have been observed across a wide range of taxa, including humans, rodents, and fish. One significant axis of behavioral variation is risk-taking, where animals displaying a greater willingness to take risks are classified as bold, while those exhibiting less inclination are characterized as shy. While biological factors are known to contribute to this variation, the underlying mechanisms are not yet fully understood. To investigate the neural mechanisms underlying these behavioral differences, we employ adult zebrafish as a model. We assess behavioral differences in zebrafish by subjecting them to the novel tank test and quantifying their exploration of the new environment. Our findings reveal that bold individuals explore a larger area of the tank and spend most of their time near the top, whereas shy individuals exhibit limited exploration and spend most of their time towards the bottom of the tank. To gain a better understanding of the neural basis of bold and shy behavior types, we developed tools for whole-brain activity mapping. We used in situ hybridization chain reaction (HCR) to detect the expression of c-fos, an immediate early gene, as a means of labeling active neurons. To visualize brain-wide c-fos expression, we combined tissue clearing technique with light sheet microscopy to generate whole brain images. For automatic detection of c-fos positive cells, we employed CellFinder, a deep learning based cell identification approach integrated into the BrainGlobe computational environment. The images were then registered to our recently created adult zebrafish brain atlas (AZBA) using advanced normalization tools (ANTs). We successfully trained CellFinder to identify c-fos positive cells with an accuracy of 96% and found that c-fos expression peaks at 15-30 minutes following exposure to a novel tank. With this approach, we identified brain regions associated with boldness.

Poster Session 2 | Poster Wall 106 | Label: PS2.106

Category: Other

Modeling autism spectrum disorders in zebrafish: social deficits, visual lateralization and cerebral asymmetry

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Cerebral asymmetry is critical for typical brain function and development; at the same time, altered brain lateralization seems to be associated with neuropsychiatric disorders. In autism spectrum disorders (ASD), studies have suggested reduced functional and structural cerebral asymmetry, reporting changes in asymmetric activation of brain structures involved in language and social processing and increased prevalence of left-handedness. Zebrafish are increasingly emerging as model species to study brain lateralization, using asymmetric development of the habenula, a phylogenetically old brain structure associated with social and emotional processing, to investigate the relationship between brain asymmetry and social behavior. We exposed 5-h post-fertilization zebrafish embryos to valproic acid (VPA), a compound used to model the core signs of ASD in many vertebrate species, and assessed social interaction, visual lateralization, and gene expression in the thalamus and the telencephalon. VPA-exposed zebrafish exhibit social deficits and a “symmetrization” of social visual laterality. We also observe changes in the asymmetric expression of the epithalamic marker leftover and the size of the dorsolateral part of the habenula in adult zebrafish. Our data indicate that VPA exposure neutralizes the animals’ visual field bias, with a complete loss of the left-eye use bias in front of their mirror image, and alters brain asymmetric gene expression and morphology, opening new perspectives to investigate brain lateralization and its link to atypical social cognitive development.

Poster Session 2 | Poster Wall 107 | Label: PS2.107

Category: Other

Quantifying the energetic cost of pollen collection

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Visiting flowers to collect pollen and nectar incurs energetic costs that pollinating insects use to inform their foraging decisions, with crucial consequences for both plant and insect reproduction. While the costs of nectar-foraging can be calculated as a function of calories ingested versus expended during collection, pollen is typically not ingested at the flower by foraging bees and therefore the costs of collection remain under-explored. Nectar-foraging bees are known to adjust flower choices according to the volume and sugar concentration of nectar available and recent studies of bumblebees have shown that individuals are willing to invest time in learning to collect nectar from flowers that are comparatively more difficult to handle, providing the sugar content is sufficiently high. The fact that pollen-collecting bees do not ingest pollen at the flower, coupled with the more complex nutritional composition of pollen, make it less obvious which currency bees might aim to maximise when foraging for this reward. While nectar-foraging bumblebees maximise the rate of energetic return to the colony, rather than individual energetic gain, do pollen-foraging bees attempt to maximise the volume of pollen collected over time, or rather some measure of nutritional quality such as protein or lipid content? Here we present the results of a laboratory study which used a combination of behavioural assays and flow-through respirometry to measure the energetic costs of pollen collection in the bumblebee (Bombus terrestris) for the first time. Flower orientation was shifted between trials to manipulate the handling requirements of both real and artificial 3D-printed flowers, to directly test how this affects the efficiency with which bees are able to extract pollen rewards.

Poster Session 2 | Poster Wall 108 | Label: PS2.108

Category: Other

Individual differences in behavior: a multi-trait study across different ant species

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Animal personality refers to consistent individual variations in behaviour over time, and personality traits correlate to form behavioural syndromes. Ant personality has been studied at both individual and colony levels, but varying methodologies hinder species comparisons. We developed a protocol to systematically characterize personality traits, and applied it on Formica fusca, Camponotus aethiops, Aphaenogaster senilis and Messor barbarus. We analysed four behaviours reflecting responses in different contexts: exploratory activity, sociability, brood care, and reaction to prey. Behaviours were assessed twice for each individual to test for repeatability over time, a critical step to define personality traits. Inter-individual differences in exploratory activity and reaction to prey were notably repeatable across species ($R > 0.3$), except respectively in A. senilis and C. aethiops. Sociability was weakly to moderately repeatable in all species (R 0.4). A behavioural syndrome between sociability and brood care was apparent in M. barbarus, reflecting intra-colony behaviours, whereas A. senilis showed a behavioural syndrome involving reaction to prey and brood care, possibly reflecting the investment in brood feeding. Our protocol is generally robust, with moderate to high repeatability for most traits and species. This protocol applies the same methodology for comparing the behaviour of different species, with the advantage of including both extranidal and intranidal behaviours. Since personality differences may be connected to the way individuals interact with their environment, our multi-trait protocol will now be used to investigate the link between personality and cognition.

Poster Session 2 | Poster Wall 109 | Label: PS2.109

Category: Other

Effect of narrowband monochromatic blue and green light on magnetic orientation of night-migratory songbirds

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Night-migratory songbirds are known for their precise navigation. For their journey they rely on several cues such as the sun, the stars, and the geomagnetic field. In recent years, evidence has suggested that magnetoreception in night-migratory songbirds use a light-dependent quantum mechanism known as the radical pair (RP) mechanism.

Behavioural experiments have suggested that wavelengths ranging from UV to green light allow birds to orient in their appropriate migratory direction and that their magnetic compass is affected by radiofrequency (RF) fields. In recent years, it was shown that weak broadband RF fields has a stronger effect on the magnetic orientation behaviour than single frequencies. Cryptochrome 4 (Cry4) is considered as the most likely putative magneto-receptor because it binds FAD and forms radical pairs (RP) up on photoexcitation and Cry4 is found in the avian retina. Furthermore, in vitro experiments show that European robin Cry4 can form magnetically sensitive RPs on blue light activation.

Although considerable progress has been made towards understanding avian magnetoreception, the orientation of migratory birds under green light is still a mystery. FAD in the avian retina exists as FADox with an absorption spectrum up to 500nm. Green light falls outside this range. Further behavioural studies have claimed that magnetic orientation in green light lasts only for an hour after being pre-exposed to white light.

Our study aimed to test the magnetic orientation under much better controlled, narrow spectrum, monochromatic blue and green light. We tested Eurasian blackcaps (Sylvia atricapilla) under very narrowband monochromatic blue and green light and the preliminary results show a disorientation under green light and appropriate seasonal orientation under blue light. Considering the many reports of green light orientation, we are currently in the process of internal replication.

Poster Session 2 | Poster Wall 110 | Label: PS2.110

Category: Other

Comparisons of Neurobiological Characteristics of Vigilance in Wild and Laboratory Rattus norvegicus

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Although 95% of all preclinical animals in biomedical research are laboratory rodents (Hickman et al., 2016), little is known about how selectively-bred laboratory R. norvegicus compare to their wild counterparts. Approximately 75 years ago, neurophysiologist Curt Richter reported hypertrophied adrenal glands in wild rats compared to laboratory rats, suggesting increased stress responsivity in the wild rodents (Richter, 1949). Recently, our lab confirmed this finding when we reported that wild rat adrenal glands were approximately 300% larger than the lab rats. Additionally, wild rat neuronal density was higher in the cerebellum (Jacob, 2022). Noting excessive threats in the rats' natural habitat, in the current study we explored brain areas associated with vigilance and adaptive responses in wild rats. Male and female wild rats (R. norvegicus) were trapped in an urban section of Richmond, VA (USA) and compared to sex and weight-matched laboratory rats obtained from Inotiv, INC (n=4 for each male group; n=7 for each female group). Following brain extraction, brains were post-fixed and processed for glucocorticoid and mineralocorticoid receptor (GR; MR) immunoreactivity in the hippocampus (CA1 and CA2 areas). Thionin was also used to quantify neural and glial cells in relevant cortical areas (i.e., the pyriform, auditory, somatosensory, and motor cortical areas). The lateral habenula was investigated due to its role in coping appraisal. Two-way ANOVAs revealed a significant main effect for habitat context in several brain areas, including increased GR-ir in CA2 hippocampus, increased neurons in the lateral habenula, increased glial density in the pyriform cortex, and increased neurons in the auditory cortex of wild rats.

Poster Session 2 | Poster Wall 111 | Label: PS2.111

Category: Other

Comparative Investigations of Wild and Laboratory Rattus norvegicus: Behavioral and Neurobiological Insights

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Considering the selective breeding and artificial environments of traditional laboratory rodents, it is important to consider the degree to which laboratory rodent neurobiological characteristics are representative of their wild counterparts. A previous comparison of wild and laboratory rats (Rattus norvegicus) revealed heightened stress responsivity in the wild rats [i.e., higher corticosterone fecal metabolites, heavier adrenal glands; (Jacob et al., 2022)]. In the current study, wild rats were kept in large outdoor cages for approximately five days so that behavior could be assessed. Specifically, wild male and female rats were trapped in urban habitats in Richmond, VA, USA, and their weight-and-sex-matched laboratory counterparts were obtained from Inotiv, INC (n=3 for each group; N=12). Motion-activated cameras were installed in the outdoor and laboratory facilities so that behavior could be assessed. Results indicate that the laboratory rats retrieved more food rewards during the problem-solving challenge task ($p = 0.035$); however, no differences were observed in nest building responses. Following behavioral observations, animals were anesthetized so that the brains could be removed for histological preparation to quantify markers of neuroplasticity (e.g., BDNF and doublecortin), as well as neuronal structure (using Golgi histology). The brain data and analyses are forthcoming. In addition to the problem-solving differences observed between the wild and lab rats, informal observations indicated heightened defense responses in the wild rats that will be investigated in future studies. An enhanced understanding of these differences will inform researchers of the representative nature of investigations with laboratory rats---information that is important in determining the translational value to other species such as humans.

Poster Session 2 | Poster Wall 112 | Label: PS2.112

Category: Other

Host social behavior shapes microbiome composition and function

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Growing recent evidence has established that host-associated microbes are instrumental for host development and health. Understanding the drivers of microbiome assembly, composition and variation is thus critical for promoting individual and societal health. Recent ecological discoveries indicate that inter-host dispersal of resident microbes via social interactions contribute to microbiome assembly. Although mechanisms of microbial transmission between individuals have been investigated in the context of infectious diseases, little is known about social transmission of beneficial microbes and the impacts of socially transmitted microbiomes on host health. We take advantage of the zebrafish model to carry out fine manipulations of social behaviors and microbial associations, allowing us to isolate the social component of microbiomes, established through social interactions, and its role in host health. We characterize early stages of zebrafish social behavior and uncover novel social interaction patterns associated with microbial transmission. We demonstrate that physical contact facilitates direct transmission of specific zebrafish-associated beneficial bacteria enriched in social microbiomes. Conversely, bacteria that exhibit traits found in pathogens, such as high motility, can overcome host social isolation to ensure their own dispersal. We also find that social interactions are often asymmetrical, resulting in non-reciprocal microbial transmission, suggesting that individual hosts may benefit or not from specific microbiome functions depending on their position within the social network. Finally, we show that microbes enriched in social microbiomes promote host neuro-immune maturation, suggesting that social microbiomes exhibit features with selective advantages for the host, likely resulting from co-evolution between the host and its associated microbes.

Poster Session 2 | Poster Wall 113 | Label: PS2.113

Category: Other

A non-invasive method for describing and classifying internal states during immobility in insects

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Animals spend major parts of their lives being seemingly immobile: sleeping, recovering from activity, awaiting prey or hiding from predators. These states require very different internal tuning. Although we are moving towards understanding neural mechanisms in naturalistic contexts, immobile behaviours have received little attention despite being a fundamental part of the natural behavioural repertoire. Describing and distinguishing between immobile states non-invasively is a major challenge because of the lack of movement with which to associate neural recordings. This difficulty is especially acute for species, such as insects, that are unable to move their compound eyes.

Here we present work on the ground beetle, Nebria brevicollis, in a novel setup which combines high-resolution videos with respirometry. We developed an air-tight trackball system, that allows simultaneous measurements of metabolic rate and continuous recordings of micro-movements of mouthparts, quantified by using pose-estimation software. By combining the behavioural output with our metabolic rate measurements, we provide a description and classification of internal states during immobility.

We demonstrate how the application of our non-invasive method can be used to infer internal states of insects during immobility. We anticipate that the outcomes of this research could lead to identification of currently undescribed states or sub-states (such as sleep stages), as well as pinpointing transitions between states. Furthermore, we hope our approach and method will encourage other neuroethologists to incorporate broader physiological measures into their work, providing a starting point from which to gain new insights into the evolution of immobile states.

Poster Session 2 | Poster Wall 114 | Label: PS2.114

Category: Other

The footprint of anthropogenic noise on tunicate neuroethology

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Sound is a sensory cue that can travel the farthest in the sea and it is used by numerous marine organisms to perceive their surroundings and interact with conspecifics or other species. Marine soundscapes are rapidly evolving as a result of increasing anthropogenic activity. Thus, there is growing interest in studying the impact of anthropogenic noise on the nervous system and behavioral repertoire of marine organisms.

We study the impact of anthropogenic noise on the nervous system of marine invertebrates using Oikopleura dioica and Ciona intestinalis. These two tunicates have miniature nervous systems, which endow them with surprisingly rich behavioral repertoires and multisensory abilities. They are equipped with cells homologous to vertebrate hair cell mechanoreceptors raising the question of their ability to sense, and therefore be affected by noise.

Using the markerless pose estimation tool DeepLabCut we tracked Ciona and Oikopleura larvae and adults performing ethologically relevant behaviors, while being subjected to ambient noise or anthropogenic maritime traffic noise sampled from different sites across Europe by the DeuteroNoise consortium. We will show how anthropogenic noise impacts the ability of Ciona larvae to use a polymodal neural circuit to identify suitable substrates for settlement and metamorphosis. Furthermore, we will present evidence of how underwater noise pollution impacts the ability of Oikopleura adults to build through a series of stereotyped behaviors, a specialized structure called the house (Oikos), which is essential for filter-feeding and protection from predators. We investigate the ability of Oikopleura adults to filter-feed and escape this house under control and noise polluted conditions. Finally, our comparative analysis in closely related species aims to reveal common mechanisms at the molecular, cellular and circuit level for nervous system/behavioral adaptation to anthropogenic noise.

Poster Session 2 | Poster Wall 115 | Label: PS2.115

Category: Other

Characterization of intersexual agonistic behavior in the weakly electric fish Gymnotus omarorum

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Agonistic behavior, present in all animal species, regulates conflict situations over limited resources. The genus Gymnotus is noted for its aggressive behavior, which becomes violent when agonistic encounters take place in confined spaces. Gymnotus omarorum is a weakly electric fish, and although it is a seasonal breeder, it shows aggression throughout the year, between males, between females, and most strikingly, between males and females, even during the reproductive period. It is a monomorphic species, there are no external sexual differences, neither anatomical nor electrical, not even during the reproductive period. Our group has extensive knowledge of the factors that modulate the agonistic behavior of this species in intrasexual dyads. Intersexual aggression is rare in nature, but its occurrence in the human species, gender-based violence, is the cause of many social and health problems.

o find experimental models in which to explore the neural bases of this behavior is of great interest, and this species is the ideal model to study them. Here we describe the agonistic behavior of intersexual dyads of Gymnotus omarorum in their locomotor and electrical displays during the reproductive and non-reproductive seasons in semi-natural conditions. In most cases the heavier animal wins, regardless of sex. The conflict was longer during the non-breeding season than in the breeding season. No differences were found in the attack rate between dominant males and dominant females, nor between subordinate males and subordinate females. In the non-breeding season, only few transient electrical signals were observed, in contrast to the breeding season, when these signals are abundant. No differences were found in the duration of the conflict when females won from when males won. Surprisingly, there are no differences between the way females and males fight even in the reproductive period. We want to investigate the differences between this type of aggression and courtship.

Poster Session 2 | Poster Wall 116 | Label: PS2.116

Category: Other

DH44 modulation of defensive behaviors in Drosophila melanogaster

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Adaptive responses to external threats are essential for animal survival. Prey animals decide flexibly whether to freeze or to flee, based on several factors including distance and speed of the approaching threat, the context in which it occurs and the prey's state.

Great progress has been made in understanding neural circuits mediating threat detection and the execution of immediate defensive behaviors, but how the threat leads to sustained changes in physiology and behavior and how those may promote adequate responses to subsequent threat is far from being understood. Neuromodulation could play a fundamental role, considering that it is thought to drive state-specific neural circuit function. In mammals corticotropin-releasing factor (CRF), a neuropeptide that coordinates stress responses, plays a fundamental role in regulating defensive state. The diuretic hormone 44 (DH44), the fly homolog of CRF, is well known to mediate stress responses to e.g. desiccation, starvation, heat, CO_2, however its involvement in defense upon predatory threats is still unknown and unexplored. To study the role of DH44 in regulating defensive responses, we use the high-throughput behavioral paradigm developed in our lab, where flies are exposed to repeated looming stimuli in enclosed arenas, to which they respond with a variety of transient and sustained defensive responses, while genetically manipulating neuronal activity and DH44 signaling in identified populations of neurons. We show that downregulation of DH44 and of its receptors decreases freezing. Now we are analyzing in detail the behavioral responses as well as dissecting the neural circuits. Our findings demonstrate the involvement of DH44's in defensive behavior of fruit flies, highlighting the functional similarity with that of CRF, its mammalian homologue. Given that defensive behavior and DH44 are conserved from insect to mammals, we expect to find generalisable principles of organization governing this process.

Poster Session 2 | Poster Wall 117 | Label: PS2.117

Category: Other

Who Are You Calling A Shrimp? Evaluating Aggression, Boldness and Behavioral Strategies in Invasive Stomatopods

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Animal behavior can have enormous ecological impacts on a species' ability to invade new habitats and compete for resources. In particular, behavioral differences in traits such as aggression and boldness have been shown to play an important role in the interactions between native and invasive species. Previous studies have shown two behavioral strategies that contribute to the success of an invasive species: behavioral syndromes and the ability to learn. Stomatopods are marine crustaceans recognized for their spectacular visual systems and forceful raptorial appendage strikes. While behavioral comparisons have been made between stomatopod species through territorial contests, the differences in aggression or other behavioral traits within a smasher species, specifically in the context of invasive species and success in a non-native range, have not been thoroughly studied. This study aims to characterize the boldness and aggression within and among individuals of the invasive stomatopod Gonodactylaceus falcatus to determine if learning ability or a boldness-aggression behavioral syndrome is present, to document the overall behavioral variation of this species, and to gain insight into how these behaviors may affect intraspecific interactions. Following individual assessments of boldness and aggression, randomly selected pairs will be placed in territorial contests to analyze the impacts of individual behavioral traits on fighting sequence and contest outcome. Replicates of all three assays are performed to address questions surrounding these behavioral strategies through comparisons of within-individual responses. Studying invasive individuals in multiple aspects of behavior will allow for inferences regarding their pathway to success and provide a repeatable experimental setup for future tests of native species behavior to understand how this invasive species may impact its sympatric native counterpart.

Poster Session 2 | Poster Wall 118 | Label: PS2.118

Category: Other

Leaderless consensus decision-making determines cooperative transport direction in weaver ants

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Animal groups need to achieve and maintain consensus to minimise conflict among individuals and prevent group fragmentation. An excellent example of consensus challenge is cooperative transport: multiple individuals cooperate to move a large item together. This behavior, regularly displayed by ants and humans only, requires individuals to agree on which direction to move in. Unlike humans, however, ants cannot use verbal communication but most likely rely on private information and/or mechanical forces sensed through the carried item to coordinate their behaviour. We investigated how groups of weaver ants (Oecophylla smaragdina) achieve consensus during cooperative transport using a tethered-object protocol, where ants had to transport a prey item tethered in place with a thin string. This protocol allowed the decoupling of the movement of informed ants from that of uninformed individuals. We show that weaver ants pooled together the opinions of all group members to increase their navigational accuracy. We then confirmed this result using a symmetry-breaking task, where we challenged ants with navigating an open-ended corridor. Weaver ants are the first reported ant species to use a 'wisdom of the crowd' strategy for cooperative transport, indicating that the consensus mechanisms used by ant species differ according to their ecology.

Poster Session 2 | Poster Wall 119 | Label: PS2.119

Category: Other

Untangling wires: uncovering the organisational architecture of neuronal networks in the posterior slope region of the Drosophila brain

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Despite more than a century of research on the Drosophila melanogaster brain, studies of connectivity and biological function have focused extensively on a small number of brain regions involved in sensory processing, learning and memory as well as motor control. However, except for these, much of the Drosophila brain remains poorly understood regarding its functional connectivity. Systematic mapping and classification of the neuronal circuits in as yet unchartered, so-called "terra incognita" regions are needed to gain a greater understanding of the Drosophila brain functions and to elucidate upstream and downstream connections of individual neurons. Neurons have unique projection patterns, which conceivably influence their information-processing pathways. It is therefore critical to understand how neurons with similar or different projection patterns are organised, and how their connections are arranged in circuits. The posterior slope (PS) is one example of a previously unexplored neuropil in the Drosophila brain.

To understand this region in more detail, both at the level of organisation of neurons, and connectomically, we have devised a hierarchical classification system to organise and map neurons within this neuropil. We have now classified PS-typed neurons into families, orders and classes using the flyEM (electron microscopy) hemibrain dataset in combination with light microscopy images. This classification system has enabled us to build a morphological "map" of this neuropil.

Further to this, we show that PS-associated neurons (those neurons which partially innervate this region but are not typed as PS neurons) include sensory neurons and also include many descending neurons. Therefore, this implicates the PS as a region that integrates sensory signals to likely inform behaviour.

Poster Session 2 | Poster Wall 120 | Label: PS2.120

Category: Social behavior and neuromodulation

The impact of parental care quality on Mimetic poison frog tadpole behavior and physiology

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Mimetic Poison Frog (Ranitomeya imitator) tadpoles rely on their parents for nutritional needs. This biparental species coordinates sex-specific parenting tasks through vocal communication, including guarding, transportation, and feeding. Tadpoles induce maternal provisioning of unfertilized egg meals by begging using bodily vibrations directed at a caregiver. Whether tadpoles recognize their parents or display begging to any potential caregiver, related or not, remains poorly understood. To test this, we exposed R. imitator tadpoles to related and unrelated males and females and measured begging time and time near the stimulus. We found that tadpoles beg more to their biological mothers than unrelated female caregivers, but exhibit no preference for biological fathers. Next, we found that offspring care relies on interparental synchrony and that the degree of synchrony between parents impacts how often tadpoles receive care. Thus, we investigated how the quality of parental investment impacts begging, parental preference, and boldness. While less parental care leads to more overall begging, only tadpoles raised by high-investment parents exhibit significant preference for their mothers. Additionally, tadpoles with more invested parents were more active around a novel stimulus (i.e. bolt). Together, our data indicate that the quality of parental investment drives parent recognition and preference, and high parental investment produces bolder tadpoles. Next, we aim to identify what parts of the brain are most sensitive to the level of parental investment. We are currently examining the mitochondrial activity across brain regions in tadpoles with high and low parental care using a COX-1 stain to determine levels of neurogenesis across socially relevant brain regions. These experiments fill an important gap in our understanding of how parental decisions impact the underlying mechanisms of offspring behavior that determine their survival rate.

Poster Session 2 | Poster Wall 121 | Label: PS2.121

Category: Social behavior and neuromodulation

Epigenetic mechanisms of social plasticity in poison frog tadpoles

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For most social species, early-life isolation instigates a developmental cascade that results in social dysfunction. Some poison frogs (Family Dendrobatidae) place tadpoles within isolated nurseries while others place them in ponds as a group. We investigated the impact of solitary versus communal rearing on the morphology, behavior, and neural gene expression of the brilliant-thighed poison frog, Allobates femoralis. Our findings revealed that tadpoles raised in isolation grow larger and exhibit fewer retinotectal projections compared to their group-reared counterparts. We also measured tadpole behavior in open-field and social-place preference assays. No behavioral differences were observed between conditions during the open-field test. However, group-reared tadpoles preferred spending time near social stimuli, while isolated animals did not present a preference. To investigate epigenetic mechanisms that might drive this switch, we performed small RNA-seq experiments with neural tissue. We found differential expression of microRNAs targeting neuronal nitric oxide synthase (nNOS), an enzyme that produces nitric oxide (NO), a retrograde neurotransmitter with brain-wide influence on neural connections. To discover whether isolation influences NO production and whether that production is spatially correlated with candidate miRNA expression, we performed combined immunohistochemistry and in situ hybridization (IHC/ISH) for nNOS and its putative miRNA regulators with cryosectioned brain tissue from isolated and group-housed tadpoles. Future directions will involve in vivo miRNA manipulations and behavioral/molecular assays to establish a causal link between miRNA patterns and context-dependent behavior. Understanding the interplay between these two regulators takes us another step closer to characterizing the systems of neural mechanisms underlying behavioral plasticity.

Poster Session 2 | Poster Wall 122 | Label: PS2.122

Category: Social behavior and neuromodulation

Exploring neuroendocrine mechanisms underlying sex differences in territorial aggression: the case of the Dyeing poison frog

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Territorial aggression mediates primary access to resources that are essential for survival and fitness in many species. While androgens have long been associated with aggression in male vertebrates, females also show androgen-mediated aggression, and in several species female androgen levels are positively correlated with those of males. This observation has led to the hypothesis that sex differences in androgen-mediated traits might be a product of correlated selective responses between sexes. However, later studies challenged this view by hinting that selection acts independently on aggressive behavior in each sex based on their mating and parenting strategies. Using simulated territorial intrusions and non-invasive methods to measure androgens, we explored the hormonal basis of sex differences in territorial aggression in the Dyeing poison frog, Dendrobates tinctorius. This species, characterized by polygyny, male parental care and aggressive territorial behavior in both sexes, provided a unique model for our study. Our findings indicate that both sexes display comparable levels of aggression in response to territorial challenges, yet without the predicted androgen responsiveness. Interestingly, female androgen levels decreased after territorial aggression, suggesting alternative hormonal mechanisms that deplete androgens during social competition. Our current work uses pS6-immunoreactivity as a marker for neural activity to identify brain regions associated with territorial aggression in males and females. With this ongoing research we will shed light on how evolutionary shifts in behavior emerge from changes in neuroendocrine architecture.

Poster Session 2 | Poster Wall 123 | Label: PS2.123

Category: Social behavior and neuromodulation

Whole-brain representation of multimodal courtship cues

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Animals evolved a wide range of approaches to attract opposite-sex partners, and most of these strategies rely on the integration of courtship cues across multiple sensory modalities. In rodents, male mice attract females using a combination of olfactory (i.e. pheromones and molecules contained in their urine and scents) and acoustic (i.e. ultrasound vocalizations, USVs) cues. During mate selection, females perceive and integrate multimodal information to drive mate choice. Despite the role of multisensory communication in courtship behaviors has been studied from an ethological point of view, the neuronal mechanisms underlying the integration of courtship cues is largely unknow. Moreover, to what extent olfactory and acoustic stimuli are integrated to drive mate preference is unclear. By combining acute sensory stimulations with whole-brain immunolabelling of immediate early genes (cFOS), iDISCO tissue clearing, and light sheet microscopy, here we investigate the neuronal responses to courtship cues across the whole brain. Females mice were first exposed to either olfactory, acoustic or the combination of the two stimuli. Brains were then processed and activated brain areas were mapped by looking at cFOS expression across all regions. Preliminary data indicate that olfactory courtship cues are the main driving force for neuronal recruitment, while USVs only activate locally confined circuits in primary auditory areas. Despite the co-presentation of sexual odors and USVs recruit brain areas largely overlapping with those activated by odors only, we identified a subset of cortical and hypothalamic areas which were activated only by the exposure to multimodal courtship cues. Overall, our data suggest that exposure to multisensory courtship displays activate brain areas beyond those recruited by each single modality. Future experiments will aim to use functional approaches to precisely characterize neuronal responses in identified multimodal brain areas.

Poster Session 2 | Poster Wall 124 | Label: PS2.124

Category: Social behavior and neuromodulation

Evoked vocal responses in a frog are modulated by dopamine

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When vocalizing, many animals engage in decision-making processes that integrate social context and environmental information. The midbrain dopaminergic system is thought to be important for this process, and is relatively conserved across vertebrates. For instance, in songbirds such as zebra finches and European starlings, modulation of dopamine release appears to contribute to social context-dependent changes to song. However, relatively little is known about how similar processes may occur in other taxa, particularly the highly vocal anurans (frogs and toads). Here, we conducted a series of experiments in which we treated wild-caught male túngara frogs with a general dopamine agonist (apomorphine) to assess their vocal decision-making in response to various auditory stimuli, in addition to effects on motor performance and motivation. We found that this agonist enhanced the specificity of frogs' evoked vocal responses to auditory stimuli as evidenced by decreased response latencies and lower call overlap with the stimulus. These results highlight a role of dopaminergic circuits in modulating vocal outputs based on social inputs within a species of basal tetrapod.

Poster Session 2 | Poster Wall 125 | Label: PS2.125

Category: Social behavior and neuromodulation

Contributions of Different Brain Areas to Social Recognition in Mice

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Social behaviours are ubiquitous among animal species and help organisms increase their chances of survival by enabling cooperation. Successful cooperation between animals requires learning and recognising the degree of familiarity between animals and selecting the appropriate actions to engage. Here we sought to find novel approaches to characterise social recognition in mice and begin to better understand how the brain processes and integrates information about familiarity. We devised a freely moving experimental paradigm to define how laboratory mice (mus musculus) learn to recognise each other. We established familiarity between two animals by allowing a mouse to freely interact with a novel conspecific over multiple sessions. Afterwards, we introduced the experimental mouse to another novel animal to compare social behaviour between familiar and novel pairs. Tracking multiple animals using pose-estimation algorithms (SLEAP, Pereira et al. 2022), we showed that interaction time decreases as animals become more familiar with each other, which is in line with previously reported studies using similar protocols. Further we performed lesions of the medial prefrontal cortex (mPFC), bed nucleus of the stria terminalis (BNST), basolateral amygdala (BLA) and the ventral hippocampus (vHPC) in different cohorts of mice. Our preliminary results suggest that lesioning these areas changed distinct aspects of social interactions including increased aggression (mPFC), decreased interaction time (BNST/BLA) or a lack of distinction between novel and familiar animals (vHPC). Going forward we will exploit the richness of our social behaviour dataset by analysing behavioural sequences and motifs using unsupervised classifiers to better quantify social recognition and understand the unique contributions of different brain areas.

Poster Session 2 | Poster Wall 126 | Label: PS2.126

Category: Social behavior and neuromodulation

Female vocal feedback promotes song learning in male juvenile zebra finches

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Social interactions promote vocal learning but little is known about how social feedback affects the vocal learning process and its underlying neural circuitry. To address this issue, we explored song imitation in juvenile male zebra finches raised either in the presence or absence of females. By tutoring juvenile zebra finches with a controlled tutor song and simultaneously tracking their song learning progress in relation to cooccurring female vocalizations, we found that the company of an adult female leads to a more accurate spectral and temporal copy of the tutor song. In cases when the number of female calls correlated with song practice throughout development, juveniles exhibited a tendency to sing a song more closely resembling the tutor song hinting towards the possibility that females may provide practice-specific feedback. To decipher whether female vocal feedback has an impact on the neural activity within the song learning pathway, we performed intracellular recordings of HVC projection neurons in listening and singing zebra finches. In juvenile zebra finches, we found that female vocalizations can modulate neural activity in HVC during passively listening and singing. In contrast, in singing adult zebra finches female calls do not have an impact on the singing-related neural activity pattern. Interestingly, we found female call-evoked responses outside of the context of singing to persist after development suggesting an age-independent mechanism for the representation of behaviorally relevant vocal feedback. These results highlight the contribution of female vocal feedback to developmental song learning and how vocal input from a conspecific other than the tutor can influence the neural circuit involved in song learning and production.

Poster Session 2 | Poster Wall 127 | Label: PS2.127

Category: Social behavior and neuromodulation

Visual-Motor Circuits for Action Coordination in Schooling Fish

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Collective behaviors enhance individual survival by allowing individuals to share information about predators and prey, and have been shown to emerge from simple inter-animal interactions. Despite extensive behavioral studies and computational simulations, little is known about the neural processes of the individuals engaged in such cooperation. Our lab studies Danionella cerebrum, a highly social miniature fish species in which we have identified vision as the primary sensory basis for their coordinated schooling behavior. I am studying how Danionella collectively escape from both mechanical and visual stimuli. By manipulating the group size and the available sensory information (visual or mechanical), I aim to understand how the avoidance actions of social partners modify an individual's responses toward the perceived threat. I present visual stimuli that mimic approaching predators, as well as schooling or escaping social partners to head-tethered Danionella in virtual reality with concurrent two-photon calcium imaging. I hypothesize that there are partially overlapping visuomotor circuits that allow individual fish to robustly coordinate their movement with neighbors while remaining reactive to unexpected stimuli such as visual threats or socially inferred danger. This will provide new insights into how individual-level neural computations produce coordinated group-level behavior.

Poster Session 2 | Poster Wall 128 | Label: PS2.128

Category: Social behavior and neuromodulation

How do brains balance information for collective behavior?

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A major question in neuroecology is what information do animals use to behave, especially during collective behavior? The null hypothesis is that there is a single threshold that, once reached, causes an animal to perform a job. While this hypothesis has been explanatory for many behaviors, some behaviors do not fit this threshold. The alternative hypothesis appears to be true for a specialized honey bee thermoregulatory behavior. During hot weather, honey bees manage their colony temperature to 35C by fanning – where bees stand at the opening of their colonies and fan their wings. This circulates air and cools the colony, maintaining the ideal temperature for brood development. We tested the hypothesis that temperature is the only information honey bees use to fan by heating them in isolation. Isolated bees rarely fan. When in a group with other bees, individuals are more likely to fan. With group size, likelihood of fanning increases, while the temperature at which fanning begins decreases. This indicates there is additional, likely social, information that honey bees are utilizing to perform the fanning behavior. Going forward, my lab aims to explore the neuroethology of balancing social and thermal information to perform collective behavior.

Poster Session 2 | Poster Wall 129 | Label: PS2.129

Category: Social behavior and neuromodulation

Neuromodulator receptor gene expression in electrosensory brain regions varies across species of electric knifefishes

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Neuromodulators, including monoamines and neuropeptides, modulate signal production and perception. Motor and sensory circuits regulating communication often express neuromodulator receptors, which allow neuromodulators to differentially modulate the production and perception of signals. Whether expression of neuromodulator receptor genes in these circuits varies across species that vary in sociality, however, is less explored. Weakly electric apteronotid knifefishes are an excellent system in which to investigate neuromodulation of sensory systems. Knifefishes vary in social behavior, electric signal perception is often sensitive to neuromodulators, and the neural pathways controlling electrocommunication are well-characterized. The electrosensory lateral line lobe (ELL) and the dorsal torus semicircularis (TSd) process electric signals. We used qPCR to quantify expression of several neuromodulator receptor genes (serotonin, dopamine, vasotocin, isotocin, substance P) in the ELL and TSd across three species that vary in sociality: territorial Apteronotus albifrons, semi-social Apteronotus leptorhynchus, and gregarious Adontosternarchus balaenops. We housed fish in isolation, same-sex pairs, or opposite-sex pairs overnight and collected brains the next morning. Gene expression did not vary consistently across sex or social context. Expression of every neuromodulator receptor gene of interest, however, varied significantly across species in both the ELL and TSd. Territorial A. albifrons and semi-social A. leptorhynchus had greater expression of many receptor genes than social A. balaenops. These results suggest a greater potential for neuromodulation of sensory processing in species that have less frequent, but more intense or unstable social interactions. Correlated gene expression was also limited both within species across context and across species within context. Gene expression in electrosensory circuits is, therefore, highly variable, and evolutionary labile.

Poster Session 2 | Poster Wall 130 | Label: PS2.130

Category: Social behavior and neuromodulation

Maternal separation affects social vocalizations in juvenile and adult Mongolian gerbils (Meriones unguiculatus)

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Childhood neglect increases the risk of developing mental and social communication disorders. Maternal separation (MS) is frequently used as a stress model in rodent studies to examine behavioral responses to stressful conditions. This study measured how MS affects social interactions in juveniles and later adults using Mongolian gerbils (Meriones unguiculatus), which produce a rich vocal repertoire during social interactions. Gerbils were subjected to MS for 24 h from postnatal days 7 to 8. After MS, pup isolation calls were recorded while they were temporally separated from their mother. As a result, in MS group, the number of vocalizations decreased by about half, and the acoustic analysis revealed the frequency difference between the start and end points of vocalizations became smaller, and frequency modulation also became smaller. Subsequently, after experimental subjects reached adulthood, each one was paired with unfamiliar subjects and housed together for 5 h in a novel observation cage to measure their social ability. Control pair vocalized less frequently and formed a huddle relatively quickly and remained in it for the longest period, while MS pair barely huddled through experimental period. Conversely, Mix pair, consisting of a MS and a control animal, vocalized most frequently, and huddle period gradually increased during 5 h observation period. MS gerbils' vocalizations had minor frequency modulation compared to the control, with a smaller frequency difference between the start and endpoints. These results indicate that MS deteriorate adult's ability to establish amicable relationships with conspecifics. In addition, MS simplifies vocalizations in pups and the effect persists into adulthood, and the acoustical change may contribute the deterioration of their social ability. Furthermore, the increase of huddle period observed in Mix group may indicate that social interaction with normal (i.e., control) individuals may amend the social disorder.

Poster Session 2 | Poster Wall 131 | Label: PS2.131

Category: Social behavior and neuromodulation

Interactions between social experience and alcohol sensitivity in crayfish

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The effects of social isolation on physiological and mental health are detrimental, and the relationship has emerged as a new health epidemic. A hallmark of social isolation is an increase in drug use, including alcohol. However, the interplay between social isolation and alcohol (EtOH) in the nervous system remains poorly understood. Our lab uses crayfish to study how housing conditions (i.e., isolation vs. group-housing as well as changes in isolation space) affect nervous system structure and function, and how the differences in social history translate to changes in EtOH sensitivity. Using acute EtOH exposure as a proxy to uncover socially-mediated effects, we found that social isolation significantly delayed all stages of behavioral intoxication, and this finding was paralleled by reduced excitability of individual neurons in social isolates. To better understand the underlying mechanisms, we first focused on changes in GABAergic and serotonergic systems in an identified neural circuit after social isolation. We identified receptor subtype candidates for both neurotransmitter systems, which are likely to experience neuronal redistribution and thus contribute to the observed differences in EtOH sensitivity. More recently, we found evidence for isolation effects on the cholinergic system, specifically presynaptic muscarinic receptors (mAChRs), which have inhibitory function and provide additional targets for EtOH to suppress neuronal excitability. Interestingly, we also found that small changes in isolation conditions affected EtOH sensitivity. Isolation in larger spaces produced a major suppression of EtOH sensitivity compared to smaller isolation spaces, and this effect was eliminated by injection of scopolamine, an antagonist at mAChRs, before EtOH exposure. Together, our results suggest that different conditions of social isolation cause reorganization of neuronal receptors in crayfish neurons, which underlies the behavioral changes in EtOH sensitivity.

Poster Session 2 | Poster Wall 132 | Label: PS2.132

Category: Social behavior and neuromodulation

Neural adaptations in parasitic ants and their closely related hosts

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Patterns of investment in neural circuitry may reflect an animal's behavioral repertoire and the corresponding sensory and cognitive demands.
Social parasitism is a phenomenon where certain species exploit the social structures of other species, often without providing any reciprocal advantages. Social parasites therefore may have reduced behavioral repertoires and thus reduced cognitive requirements in comparison to their hosts. The ant Aphaenogaster tennesseensis is a temporary social parasite, which means that the queen infiltrates colonies of their closely related host, Aphaenogaster picea, relying on the host to do all the work of maintaining the colony. We performed neuroanatomical analyses comparing these two species to examine how neural adaptations may contribute to the success of parasitic behavior, host defense, and the social interactions between the two species. We also looked at dopamine expression across these two species to better understand how this neurotransmitter system may support a parasite and host lifestyle. Here, I will discuss how variation in volumes of functionally distinct brain regions and dopamine cell expression may reflect this parasite-host relationship.

Poster Session 2 | Poster Wall 133 | Label: PS2.133

Category: Social behavior and neuromodulation

Increased aggression after the experience of maternal separation in adult Mongolian gerbils (Meriones unguiculatus)

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Child abuse and neglect harm brain development and raise the risk of social communication disorders. Maternal separation (MS) is a stress model in rodent studies, exploring behavioral responses to early postnatal stress. This study used the resident-intruder test to examine how MS impacts adult social behavior in Mongolian gerbils (Meriones unguiculatus), known for their rich vocalizations during territorial interactions. A total of 40 gerbils, 20 normal and 20 MS groups, receiving 24-hour MS from postnatal days 7-8, were used. In the resident-intruder test, a resident gerbil was acclimated to the experimental cage for one week to make it territorial. After acclimation, the intruder was placed in the resident subject's cage for 10 minutes, during which its behavior, vocalizations, bites and latency were measured. In addition, two types of frequency-modulated (FM) calls were observed: short-bent upward FM (prosocial call) and downward FM (agonistic call). They are often observed when they are in contact with other individuals and when they are wary of other individuals, respectively. Results showed that in the control group, males and females showed low aggression, with many not emitting agonistic calls. Conversely, the MS group exhibited heightened aggression in both sexes. Additionally, the frequency of prosocial call decreased in both males and females, with a significant trend in males. The frequency of agonistic call increased in both sexes, with a significant difference observed in females. After resident-intruder test, c-fos brain staining was conducted and showed that increased neural activity in the paraventricular hypothalamic nucleus (PVN) of the MS group, while no difference was seen in the ventromedial hypothalamic nucleus, ventrolateral part (VMHvl). These results suggest that MS affected social interactions, shifting the gerbils' prosocial behavior toward aggression and increasing their vigilance toward invading individuals.

Poster Session 2 | Poster Wall 134 | Label: PS2.134

Category: Social behavior and neuromodulation

Circadian neuropeptidomics for the analysis of coupling factors controlling multiscale behavioral rhythms in Drosophila melanogaster

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Circadian rhythms allow animals to synchronize behavioral and physiological processes to the environment. These rhythms are produced by core clock neurons in the nervous system, which generate and transmit time-of-day signals to downstream tissues, driving overt rhythms. These cells drive major activity peaks at dusk and dawn. In addition to the circadian rhythm, the fruit fly also expresses ultradian bouts of feeding. Starvation resulted in altered rhythms of activity-rest behavior. Neurosecretory neurons of the pars intercerebralis and suboesophageal ganglion, belonging to the neuroendocrine system, colocalize many neuroactive substances such as neuropeptides that regulate behavior. To identify potential peptidergic candidates that are crucial coupling factors released from central oscillator neurons in ultradian and circadian rhythms entrained to environmental zeitgebers, we used different mass spectrometric approaches. L3 larval Drosophila were subjected to fed and starved conditions (24, 48 h) at different zeitgeber times (ZT). Then, their behavior was recorded for 20 min before the end of the starvation period. For each animal, activity and rest were calculated from video recordings. Behavioral analysis revealed differences in the activity and rest of the animals between the fed and starved conditions at different ZT. After the video recording, the brain and neurohemal organ of each animal were prepared for MS analysis. Statistical comparison of the resulting mass spectra revealed semi-quantitative changes in the ion signal intensities of products of eight neuropeptide genes (sifamide, sNPF, extended FMRFamide, hugin-PK, calcitonin-like diuretic hormone-31, allatostatin-C, corticotropin releasing factor-like diuretic hormone-44, and kinin). The results of our study provide necessary input for future measurements up to the single-cell level to study the dynamics of up- and downregulation of neuropeptides underlying mechanisms regulating feeding.

Poster Session 2 | Poster Wall 135 | Label: PS2.135

Category: Social behavior and neuromodulation

Modification of taste performance by octopamine and dopamine in different honeybee species

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Honeybees are excellent models to study the molecular mechanisms underlying the social organization and rich behavioral repertoire. They are distributed across the globe because of their great behavioral plasticity and adaptation capabilities and can strongly vary in their nesting behavior, morphology, physiology and spatial distribution. Global honeybee research has focused on the Western honeybee Apis mellifera, neglecting the variation in behavioral plasticity in other honeybee species. We performed behavioral pharmacological experiments on the taste behavior in four honeybee species co-occurring in southern India. Individual taste performance correlates with numerous behavioral decisions including learning and memory and social organization. Biogenic amines can generally act as neurotransmitters, neurohormones and neuromodulators in insects. The biogenic amines octopamine and dopamine modulate taste in the Western honeybee A. mellifera. The function of these amines in Asian honeybees in unknown. We investigated how octopamine and dopamine can modulate responses to sugar in the three Asian honeybee species Apis cerana, Apis florea, and Apis dorsata, as well as the Western honeybee A. mellifera. While A. cerana and A. mellifera are cavity-nesting, A. florea and A. dorsata are open-nesting honeybees. They also differ in their foraging behavior. Octopamine significantly increased taste responses in A. cerana, A. dorsata and A. mellifera, while having no effect on A. florea. Dopamine decreased taste responses in A. cerana and A. mellifera, but had no effect on A. florea and A. dorsata. Intriguingly, responses to sugar solutions were generally very low in A. florea. Our results suggest that octopamine can increase taste responses similarly across honeybee species apart from A. florea, while dopamine can have the opposite effect in A. cerana and A. mellifera.

Keywords: Asian honeybees, Biogenic amines, Gustatory response score, Sucrose responsiveness

Poster Session 2 | Poster Wall 136 | Label: PS2.136

Category: Social behavior and neuromodulation

Singing the Praises of Dopamine Modulation: A Comparison across Cricket Species of Dopaminergic Neurons in the Context of Acoustical Communication

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Dopamine is a potent neuromodulator of behavior and can function to alter or bias many sensory and motor systems. To better understand the functions of dopamine in the context of sexual dimorphism and species-specific behavior, we focused on the behavior of crickets and their acoustical signaling/detection of a mate. In a number of cricket species, males produce songs to attract a female. In the Hawaiian Islands, however, some species of crickets have lost their ability to produce song due to a mutation affecting their wing morphology, thus preventing them from emitting sound upon wing stridulation (i.e., flat-wing morphs of Teleogryllus oceanicus). In addition, on the Big Island of Hawaii, females of a different species of crickets need to discern small differences in species-specific song when locating males that sing in groups containing multiple species (i.e., members of the Laupala genus). Might the differential patterns of dopamine investiture, especially in neural regions processing acoustical information, provide a window into the underpinnings of species-specific signaling and reception? To address this question, we examined the cytoarchitecture and numbers of dopamine-labeled neurons in the brains and ventral nervous systems of various cricket species, including the Hawaiian species discussed and the cricket species, Allonemobius allardi, found locally in the Midwest, USA. To label dopamine neurons, we used a highly specific monoclonal antibody against tyrosine hydroxylase (the rate-limiting enzyme for the synthesis of dopamine). We found that our protocols and confocal microscopy allowed for a highly detailed visualization of individual dopaminergic neurons. The nervous systems of A. allardi yielded the most reliable and robustly immunolabeled specimens. Sexually dimorphic patterns of immunolabeling were observed across species, and neurons in the prothoracic ganglion (a region for auditory processing) were conserved yet specialized across species.

Poster Session 2 | Poster Wall 137 | Label: PS2.137

Category: Social behavior and neuromodulation

Mouse ‘teachers’ and mouse ‘midwives’: behavioral mechanisms of co-parenting that improve maternal-infant survival

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Parental care helps ensure offspring health and wellness. Parenting in mammals is challenging as infants are largely helpless, needing near-constant supervision over prolonged periods of time. There is a high degree of rodent infant mortality in native habitats and in lab vivaria, from hypothermia unless pups are kept warm by a caretaker or nest. Parents must balance caretaking with other activities for their own survival, inevitably involving periods of absence from the nest.

To document the behaviors and decision-making involved in care of infant pups, we built a semi-automated, open-source system for 24/7 monitoring of homecage life, with thermal imaging, multi-camera video recording from above/beside/below the nest, and environmental controls to standardize housing conditions across cages.

We monitored 38 wild-type (WT), single-housed female mice and offspring, before, during, and after parturition, up to four consecutive litters. About half the dams had high litter survival rates (‘high-pup-survival’ dams) but the other half had little to no pups survive (‘low-pup-survival’ dams). Without intervention, low-pup-survival dams kept neglecting pups and building suboptimal nests across litters, losing nearly all offspring.

We co-housed a low-pup-survival dam with a high-pup-survival dam and her litter in between litters 2-3 of the low-pup-survival dam. We co-housed a low-pup-survival dam with a high-pup-survival dam and her litter in between litters 2-3 of the low-pup-survival dam. When the low-pup-survival dam was bred again and singly-housed, litter survival rates thereafter were consistently much higher.

We imaged parturition in WT and oxytocin receptor knockout (OXTR KO) singly-housed dams. OXTR KO dams all struggled during long parturition, with increased rates of maternal and infant mortality. Co-housing with a WT female improved litter and mother survival rates as the WT female helped withdraw pups stuck in the birth canal (due to no contractions).

Poster Session 2 | Poster Wall 138 | Label: PS2.138

Category: Social behavior and neuromodulation

The role of oxytocin in fitness: Insights from mating behaviour and reproductive outcomes in zebrafish

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In vertebrates, fitness is largely defined by female fecundity and male fertility rates. These outcomes rely on mating behaviours purposed to attract mates and elicit spawning events. However, the fitness consequences of the neural mechanisms responsible for mating behaviour remain underexplored. Increasing evidence shows that oxytocin, beyond its peripheral hormonal effects on female parturition and nurturing, also regulates mating behaviour via neuromodulatory functions in the brain. Thus, we propose that this mechanism impacts reproductive fitness by affecting male-initiated behaviour during mating. To test this hypothesis, we used genetic zebrafish models that target the expression of oxytocin producing cells in the brain. Our findings show that oxytocin regulates fecundity and fertility rates, as well as yolk and larvae size, and that this is largely attributable to male genotype. Further, responses during mating with mutant or wild-type males revealed that reproductive outcomes are driven by oxytocin's control of mating behaviour. These results will be discussed in relation to mechanisms of paternal reproductive phenotype and its impact on maternal response.

Poster Session 2 | Poster Wall 139 | Label: PS2.139

Category: Social behavior and neuromodulation

Neural correlates of emotional vocal communication in rats: involvement of anterior cingulate cortex

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Emotional vocalizations serve as crucial means of communication, conveying the internal state of the sender and eliciting empathetic responses in receivers. In rats, ultrasonic vocalizations (USVs) around 50 kHz are produced in pleasant contexts, while USVs around 22 kHz are produced in unpleasant contexts. Rats exposed to 50 kHz USVs exhibit approach behaviors, while rats exposed to 22 kHz USVs exhibit avoidance behaviors, such as freezing. These behaviors resemble empathy-like responses. While prior research on rat USVs has primarily focused on the emotional state and vocal control of the caller, a comprehensive understanding of empathy necessitates studying both the sender and the receiver.

In this study, we focused on the receiver's response to the rat USVs. Our hypothesis is that the auditory perceptual processing of 50 kHz/22 kHz USVs induces approach/avoidance behaviors by modulating the dopaminergic system. As the first step to test this hypothesis, we conducted electrophysiological experiments in both acute and chronic conditions to identify neurons that are active during the USV perception. Electrodes were implanted in the rat brain, and neural activity was measured over time during intermittent presentations of the USVs and the control stimuli. We focused on several brain regions where dopamine neurons project. In both anesthetized acute and free-moving chronic recordings, we found neurons that responded to USVs in the anterior cingulate cortex (ACC).

We also observed rapid habituation in both acute and chronic conditions. We are now investigating the relationship between behavioral decision-making and response habituation in USV communication. Eventually, we will try to integrate these findings as functions of the dopaminergic system. We aim to advance our understanding of the neural basis of empathy and shed light on the intricate mechanisms underlying emotional communication.

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Poster Session 2 | Poster Wall 140 | Label: PS2.140

Category: Social behavior and neuromodulation

Social distancing: Group behavior and the underlying neural circuits in Drosophila melanogaster larvae

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Decision-making is complex, as animals not only need to consider information based on their own perception, internal state and experience, but also social cues. Living with conspecifics can be beneficial to gain more information regarding the environment and to access better resources. However, it also increases competition for mates and food. How animals sense their conspecifics and then use this information to modulate their behaviour in a social context is not very well understood. To answer this question, we are investigating group behaviour in larvae of the model organism Drosophila melanogaster. In fly larvae, we can make use of state-of-the-art behavioural tracking methods and a rich array of genetic tools to understand behaviour and the underlying neural circuits in detail. Behavioural experiments show that Drosophila larvae avoid their conspecifics in an open arena without any food and disperse more than when they are alone. Being in a group also affects decision-making in different sensory contexts and internal states. Furthermore, social distancing is dependent on social experience during development. Preliminary genetic manipulation experiments suggest that larvae sense each other via multiple sensory systems. To better understand the social dynamics, we make use of an agent-based model which can reproduce behavioural effects. Our results will help to better understand the behavioural algorithms and neural processing mechanisms that underlie social interactions between conspecifics.

Poster Session 2 | Poster Wall 141 | Label: PS2.141

Category: Social behavior and neuromodulation

Social interactions of gerbils with different expertise levels during perceptual decision making

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Gregarious animals live, learn and adapt together. This promotes social interactions that may aid adaptation to new environmental demands. Modern behavioral neuroscience strives to understand such learning processes. However, the impact of conspecifics within a task setting has been understudied and its effects on learning remain largely unknown. To this end, we study freely moving and interacting Mongolian gerbils in an auditory discrimination paradigm (“Sensory Island Task”, SIT). We studied pairs (dyads) of same-sex littermates with distinct degrees of task expertise (“expert” or “student”).

Initially, an expert was trained individually on a sound discrimination task that required performing a specific behavior upon detecting a change in the sound stimulation to receive a food reward. Once high performance was achieved, the expert was then paired with a naive student, and actions of either animal could trigger a single food reward. After experiencing multiple dyad sessions, students exhibited high levels of performance from the first day of being tested alone, demonstrating that they had learned the task during the joint sessions.

We analyzed behavioral patterns and vocal communication during task performance. Most notably, we found that animals only vocalized in the presence of a conspecific, and that the task structure had a profound effect on the timing and nature of vocalizations. Interestingly, characteristic locomotion patterns arose during competition for food rewards after correct trials, demonstrating the emergence of distinct foraging strategies.

Together, our study provides valuable insights into social interactions between individuals during perceptual decision-making and foraging, and shed light on the effect of the presence of conspecifics during task performance and learning, in a freely moving, ecologically driven task.

Poster Session 2 | Poster Wall 142 | Label: PS2.142

Category: Social behavior and neuromodulation

A Neuronal Hub for Social Behavior in Male Drosophila

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A Neuronal Hub for Social Behavior in Male Drosophila

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During social interactions, animals rapidly translate multisensory cues from conspecifics into behavioral sequences. In male Drosophila, a dozen P1a neurons per brain hemisphere have been implicated in the integration of gustatory, olfactory, and visual cues and the gating of a wide variety of aggressive and courtship behaviors such as agonistic lunges, locomotor pursuit, and production of courtship song. Yet, the neuronal and circuit mechanisms by which the P1a population integrates such sensory inputs and sculpts ongoing behavior remain unknown. Moreover, P1a neurons comprise only a small subset of a broader sexually dimorphic pC1 lineage, for which behavioral and physiological function have not been investigated in detail. In an initial effort to comprehensively characterize the male pC1 population, we leveraged the forthcoming male Drosophila melanogaster whole-brain connectome to identify approximately 70 distinct pC1 neuronal subtypes based on morphology, regions of innervation, and synaptic connectivity. We generated over 200 genetic driver lines that tile the pC1 population for the purpose of targeted optogenetic perturbation and physiological interrogation of individual and subsets of pC1 neurons.
We designed a series of high-throughput behavioral assays and adapted an automated pipeline for animal tracking and behavioral analysis with the goal of revealing pC1 neuron-specific functions in naturalistic social interactions, visually guided social behaviors, and song production. Here, we present our initial description of the behavioral functions of discrete subset of pC1 neurons, a hub for social behavior in male Drosophila.

Poster Session 2 | Poster Wall 143 | Label: PS2.143

Category: Social behavior and neuromodulation

Circuit mechanisms flexibly regulating aggression across sexes in Drosophila

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Whether deciding to pursue a mate or attack a competitor, social interactions are critical for survival. Aggressive behaviors are innate and flexible social interactions during which continuous assessment of risk of injury and potential reward guide approach, engagement, continuation, and disengagement. However, little is known about the neuronal circuit mechanisms underlying these complex aspects of aggression.

The fruit fly, Drosophila melanogaster, constitutes a powerful model for the mechanistic dissection of such aspects of aggression due to its genetic accessibility, complete brain-wide connectome, and behaviors. Recently, we uncovered a cell type underlying persistent female aggression (Schretter et al., 2020; Chiu,...,Schretter, 2023). Through mapping this complete female aggression circuit, we found these neurons exert a large part of their behavioral effects through gating visual processing. Interestingly, male courtship pursuit uses many of the same circuit motifs suggesting common mechanisms for continuing social behaviors. As persistent aggression risks injury or death, mechanisms for conversely shutting down or disengaging are equally critical for survival. Further circuit and quantitative behavioral analysis uncovered a novel neuronal subset downregulating aggression in females and males. In addition to furthering our understanding of aggressive behaviors, this work has important implications for uncovering how the brain flexibly regulates social behaviors across sexes.

Poster Session 2 | Poster Wall 144 | Label: PS2.144

Category: Social behavior and neuromodulation

Inter-individual covariation between HPA response and stress-induced social behaviour in large-billed crows (Corvus macrorhynchos)

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Animal group-living entail resource competition among individuals varied in socio-behavioural and homeostatic characteristics. The hypothalamic-pituitary-adrenal (HPA) axis regulates homeostasis for stress coping by adrenocorticotropic hormone (ACTH) and corticosteroid (CORT) signals from the pituitary and the adrenal glands, respectively. These signals are triggered by corticotropin-releasing factor (CRF) from the hypothalamus in response to stress stimuli. A previous study in birds reported that the tendency for non-social exploratory behaviour in a novel environment covaried with CORT response among individuals. However, it remains unclear in birds how social behaviour expression relates to HPA response. This study investigated the relationship between HPA response and social behaviour expression in response to a stress stimulus, using captive groups of large-billed crows (Corvus macrorhynchos), a group-living corvid exhibiting various social behaviours. We conducted a behavioural pharmacology experiment: each bird, isolated from the group, received an i.v. CRF injection to evaluate HPA response (i.e., plasma ACTH and CORT elevation) 5 and 10 min after the stimulation. In a separate behavioural experiment, we counted the number of aggressive and submissive behaviours of each bird in a group setting for 5 min after a human intrusion into the home aviary. Individual profiles of ACTH and CORT responses by the CRF stimulation and the frequency of stress-induced aggressive and submissive behaviour were combined to analyse their covariations among individuals. We found covariations between ACTH elevation at 5 min and the frequency of submissive behaviour and between CORT elevation at 5 and 10 min and the frequency of aggressive behaviour. These results suggest that individuals with higher HPA responses are more likely to express social behaviour in response to stress stimuli.

Poster Session 2 | Poster Wall 145 | Label: PS2.145

Category: Social behavior and neuromodulation

Insights from single-nuclei transcriptomics into the evolution and neural basis of parental care in two three-spined stickleback ecotypes

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Comparative approaches have long been utilised in the field of neuroethology to understand the link between genes, brain, and behaviour. Two three-spined stickleback (Gasterosteus aculeatus) ecotypes that have diverged in parental care behaviour provide a powerful within-species “natural experiment” for gaining insight into the evolution and neural basis of parental care. The ancestral “common” ecotype provides the paternal care typical of three-spined stickleback, while the divergent “white” ecotype does not provide care but instead disperses his eggs immediately after fertilization. In this study, we examine gene expression in both the telencephalon (n=12) and diencephalon (n=12) of the two ecotypes at a single-nuclei level of resolution, comparing across two stages in their breeding cycles. In one stage both ecotypes have finished building nests and are ready for courtship, while in the other stage both ecotypes have successfully spawned with a female and are either caring for or dispersing their eggs, allowing us to examine the transition into post-spawn behaviour of both ecotypes. We use these data to identify active cell clusters via immediate early gene (IEG) expression, as well as test gene-specific hypotheses, thus taking first steps into elucidating the possible neural cell populations and genes involved in parental care and the loss thereof.

Poster Session 2 | Poster Wall 146 | Label: PS2.146

Category: Social behavior and neuromodulation

Conserved perceptual biases shape the evolutionary design of an anuran visual signal

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Understanding the evolutionary pressures driving the design of animal communication behaviors remains a fundamental challenge. Here, we investigate the influence of perceptual bias on the evolution of the foot-flag display, an agonistic visual display in frogs. The foot flag involves a male extending a back leg outwards away from its body and moving it in an arc-like motion, and the signal functions to augment aggressive communication in noisy environments. Recent research suggests that foot-flagging may have emerged as a signal due to its exploitation of a conserved perceptual bias in frogs known as the worm/antiworm feature analyzer. This perceptual mechanism allows individuals to differentiate between potential prey (worm) and threats (antiworm) based on the direction of movement relative to an object's length. Our investigation focuses on the foot-flagging frog species Staurois parvus, and we show that males perform this display in a way that creates antiworm-like leg movements that deter rival males. Additionally, our findings highlight variability in male responses to the antiworm motion, with some individuals demonstrating aggression towards it, and others demonstrating avoidance behaviors towards it. Overall, our study underscores the potential role of perceptual bias, specifically the worm/antiworm motion bias, in shaping the evolution of signal design in the foot-flag display of frogs.

Poster Session 2 | Poster Wall 147 | Label: PS2.147

Category: Social behavior and neuromodulation

Transcriptome evolution in the social brain reflects phylogeny, neuroanatomy, life history, and behavior across vertebrates

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The diversity of social behavior within and across species is astounding. We now have a basic understanding of how the brain generates context-appropriate behavior in an ever-changing world. Considerable progress has also been made towards reconstructing the evolution of the neuromolecular mechanisms that regulate and generate complex behavior (such as the vertebrate Social Decision-Making Network, SDMN), underscoring the similar roles of evolutionarily ancient fore- and midbrain nuclei as well as hormonal and neuromodulatory systems in the regulation of social behavior. In addition, similar gene expression networks can underlie the convergent evolution of social phenotypes even across distantly related taxa, suggesting the repeated and parallel deployment of conserved molecular and neural pathways. Together, these results suggest that the vertebrate ancestor alive ~450 MYA already had the neuromolecular apparatus in place to meet the challenges and opportunities imposed by fluctuating internal states and external environments (e.g., finding mates, defending resources, avoiding predators). Here, we present a phylogenetic comparative approach that integrates RNA-seq and spatial transcriptomics to uncover how transcriptome variation reflects variation in ecology, demography, and life history across 24 vertebrate species. We first reconstructed the evolutionarily conserved “core transcriptomes” of subpallial amygdala and hippocampus, two key nodes of the SDMN. We then estimated rates of expression evolution to identify gene sets associated with convergently evolved social, ecological, and life history attributes. Finally, we discovered neuroendocrine candidate genes and gene co-expression networks that reflect the variation in species attributes across vertebrates. Our novel approach begins to identify the causes and consequences of variation of an ancient brain system underlying social behavior.

Poster Session 2 | Poster Wall 148 | Label: PS2.148

Category: Social behavior and neuromodulation

Molecular evolution of the genes involved in social behaviour across Lake Tanganyika's cichlids adaptive radiation

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Many molecular pathways and genes have been suggested to play a role on social behaviour across vertebrates. However, the mechanisms by which these genes regulate social phenotypes are still far to be unravelled. In this study, we aim to find genetic signatures of evolutionary transitions associated with social recognition. For this purpose, we focus on pair-bonding, a social trait that cannot exist without the former. By using the entire radiation of cichlid species in the Lake Tanganyika, in which repeated evolutionary transitions between social phenotypes occurred, this project aims to study the mutations on the coding regions of the genes potentially involved in social behaviour and use these transitions to find associations with pair-bonding. To this end, the genes of several pathways were extracted from KEGG database, as well as other genes potentially involved in social behaviour. The gene sequences of all the orthologs and their homologues were obtained, and positive selection analysis based on dN/dS ratio was performed. To detect associations between social traits and mutations, a comparative correlation analysis for discrete variables across phylogenies was performed. Over 100 genes show signs of gene-wide positive selection, and many of them present faster evolution in pair-bonding species. Ongoing correlation analyses across the phylogeny point to weak genotype-phenotype associations that do not explain alone phenotypic diversity. Future work will address other social phenotypes (group size) and study gene expression as another key mechanism that would explain intra- and interspecific variation of social phenotypes.

Poster Session 2 | Poster Wall 149 | Label: PS2.149

Category: Social behavior and neuromodulation

Brood care in shell-dwelling cichlids is governed by independent maternal and larval timing mechanisms

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Brood care requires interaction between parents and offspring, as well as intrinsic mechanisms coordinating this behavior. Lamprologus ocellatus, a small cichlid from Lake Tanganyika, has a particularly interesting brood care strategy. Female fish of this species lay their eggs in the dark, protective confines of an abandoned snail shell, where they raise them to free-swimming larvae. We devised a laboratory paradigm that allows us to observe and analyze interactions between mother and larvae within 3D printed shells. Throughout embryonic and larval development, the mother cleans, transports, and protects the fry, eventually leading to their emergence from the nest at 9 days post-fertilization. Remarkably, this emergence time coincides with a switch in phototaxis behavior from dark preference to seeking light in the larvae. We see that removing the mother does not alter the larvae intrinsic schedule, provided fresh water is supplied inside the shell. However, we were able to delay larvae emergence by using a foster mother whose biological offspring were two days younger, indicating the foster mother is presented with a conflict between the larvae's natural emergence time and her expectations driven by an intrinsic timer. Thus, larval and maternal behaviors appear to be governed by independent internal timing mechanisms, which are normally synchronized, but can be brought into conflict by experimental manipulations. The larval intrinsic schedule can be overridden in adverse conditions, while the timing of maternal duties is unaffected by the offspring's behavior. This study sheds light on the mechanisms of parent-offspring coordination during brood care in this new model organism, providing first insights into the behavioral adaptations and development of shell-dwelling cichlids.

Poster Session 2 | Poster Wall 150 | Label: PS2.150

Category: Social behavior and neuromodulation

Social Regulation of the Stinging Behaviour of Honeybees

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Honeybee colonies exhibit a remarkable defensive strategy against large predators, employing tactics such as biting, stinging, and releasing alarm pheromones to recruit additional bees for defence. While this collective defence effectively wards off predators, it comes at the cost of the bees that sting, as they die following the act. This presents a critical trade-off for the colony: balancing defence while preserving its workforce. Therefore, we hypothesized that colony defence is regulated at both individual and group levels, with factors such as an individual's threshold and surrounding social cues influencing the decision to sting. To test this, we conducted two different experiments using the stinging assay. In the first experiment, we investigated individual stinging responsiveness by repeatedly testing the same bees in four consecutive trials. We also examined responses of bees in the presence of social cues, such as the synthetic alarm pheromone and/or another bee. Our results suggest that individual bees are consistent in their decision to sting, and this consistency is influenced by their threshold and their prior behaviour within the experiment. The social cues introduced variability into this decision, indicating that social information is valued less than self-information. In our second experiment, we investigated how group composition influences the decision of bees to sting. We categorized individuals within a group as either "aggressive" or "gentle" using the stinging assay. Then, we tested the same individuals a second time in shuffled groups of specific numbers of aggressive and gentle bees. We found that group composition is irrelevant for individual bees to make the decision to sting; however, it influences the collective output of the group. Our findings dissect individual stinging behaviour within the collective defensive behaviour, thus offering insights to study decision-making processes and paving the way for a neurobiological explanation.

Poster Session 2 | Poster Wall 151 | Label: PS2.151

Category: Social behavior and neuromodulation

Dissecting the neuronal mechanisms of mouthbrooding behaviour in the African cichlid Astatotilapia burtoni

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Parental care consists of a collection of species-specific behavioural traits that one or both parents carry out to increase offspring survival. The African cichlid Astatotilapia burtoni shows one of the most extreme forms of parental care: mouthbrooding. Mouthbrooding females hold their developing young in their mouth for two weeks, during which the female refrains from feeding. Mouthbrooding leads to the neuromodulation of brain regions known to have a role in reproductive and parental care behaviour and also in feeding and energetic circuits. This project aims to dissect the neural mechanisms underlying mouthbrooding using A. burtoni. The hindbrain vagal lobe (VL) region communicates with the internal viscera and controls mouth movements during feeding in teleost fishes, making it a candidate area for the control of mouthbrooding. Still, the temporal overlap between spawning behaviour and mouthbrooding initiation makes it hard to identify the neural mechanisms behind parental care behaviour. We took two approaches to understand the neuronal basis for mouthbrooding. (1) We profiled the neuronal activation pattern of VL and other brain regions during various aspects of spawning and mouthbrooding by measuring proxies for recent neural activity and the candidate receptor ptgfr (prostaglandin F2-alpha receptor). (2) We examined mouthbrooding behavioural traits and how they associate with specific brain activation. We confirmed that the VL is activated in females that spawn, with dense bilateral cfos expression in medial regions and sparser activation in lateral regions of the VL. Additionally, we are developing transgenic lines using the QFGal4:QUAS system to provide cell-type specific monitoring of neural activity, axonal tracing, and cell ablation. Our improvements will enable the dissection of neuronal populations responsible for mouthbrooding.

Poster Session 2 | Poster Wall 152 | Label: PS2.152

Category: Social behavior and neuromodulation

Autonomic biomarkers correlate brain activity during social behaviours in birds

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Changes in social environments cause fast behavioral responses through rapid changes in physiological dynamics. This dynamic is coordinated through series of feedback loops between central and autonomic nervous systems. Heart rate (HR) is a suggested autonomic indicator of this loop. However, correlations between HR and brain activity during complex social behaviors have yet to be elucidated.

Here we developed a method to continuously measure HR and body temperature (BT) with neuronal activity in freely behaving zebra finches. We recorded single-unit neuronal activities from the Locus coeruleus (LC), the brain locus for attention/arousal control, with simultaneous measurement of HR and BT with a small wireless biosensor. We previously reported increased LC neuronal activity during vocal communications in juvenile zebra finches (Katic et al. (2022) Nat Commun). We found a positive correlation between HR and LC activities in socially interacting zebra finches (cc: 0.7, $p>0.001$, $N=8$). Correlation in HR and BT between two zebra finches was higher when they approached or vocalized to one another (no interaction: cc (BT) = 0.497, p0.001, $N=4$). Finally, we study longitudinal BT and HR fluctuations during prosocial and cognitive development in social groups of hand-reared crows.

Taken together, our results suggest continuous measurement of HR and BT in freely behaving birds might contribute to understanding the long-term impact of social behaviors on brain function.

Poster Session 2 | Poster Wall 153 | Label: PS2.153

Category: Social behavior and neuromodulation

Higher-order social interactions shape courtship behavior in Drosophila

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Social interactions in groups of animals arise from pairwise as well as higher-order interactions. For instance, courtship is commonly considered a pairwise interaction between a single male and a single female, but third parties such as rival courters and alternative mating targets can influence the interaction. The contribution of these higher-order interactions to the social dynamics in animal groups is only poorly understood.

Here, we analyzed the courtship behavior in groups of the fruit fly Drosophila melanogaster to understand the influence of third parties and higher-order social interactions. We recorded interactions in mixed groups of 4 males and 4 females from two wild-type strains and defined a dynamical social interaction network based on the proximity and alignment of flies. We then used graph- and information-theoretic methods to characterize the interaction dynamics and the role of higher-order interactions.

Information theoretical measures show that higher-order interactions strongly shape social dynamics: Flies are more likely to form male-female pairs than expected by a maximum entropy model that only takes into account independent links between flies. Only a model with higher-order interactions is able to explain the patterns of interactions in our data.

Interestingly, the importance of higher-order interactions depended on the strain: The NM91 strain exhibited larger group sizes and stronger higher-order interactions compared to the CantonS strain. NM91 courts more but discriminates less between males and females. However, the levels of female-directed courtship in both strains are similar, suggesting that different strategies can yield the same level of mating efficiency. Our results offer a framework for studying social behavior in groups of flies to identify the underlying neural circuits with the help of genetic or anatomical manipulations.

Poster Session 2 | Poster Wall 154 | Label: PS2.154

Category: Social behavior and neuromodulation

Toward understanding the molecular basis of honey bee behaviors: functional analysis of mKast, a gene selectively expressed in the adult brain, by producing knocked-out mutants

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The molecular and neural bases in the brain underlying social behaviors of the honey bee have long been studied, but little has been elucidated about them due to the lack of effective gene manipulation methods in the honey bee until recently. We have tried to apply genome editing methods to honey bees to analyze the function of genes expressed in the brain, and have established essential techniques to produce mutant honey bees (Kohno et al., 2016, 2018). Here, we focused on a gene called mKast, which is selectively expressed in the adult honey bee brain, mainly in the optic lobes (OLs) and middle-type Kenyon cells (mKCs) in the mushroom bodies (MBs), and examined its function in behavioral regulation.

We first produced mKast knocked-out workers by artificially inseminating mosaic queens (F0), which developed from larvae hatched from embryos injected with Cas9 protein and sgRNA, with semen collected from mutant drones (F1) derived from other mosaic queens (F0). Next, we compared scRNA-seq data of OLs in the honey bee and fruit fly, and suggested that mKast-expressing OL neurons are involved in motion sensing. Consistent with this, mKast knocked-out workers showed limited antennal responses to motion stimuli, which was previously reported in the honey bee, in a direction-specific manner. We also examined the effect of mKast knockout on the ability in learning and memory by classical odor or visual conditioning to investigate its function in the MBs, and found that mKast knocked-out workers exhibited comparable learning and memory abilities to WT workers. Combining the results of immunohistochemistry of mKast using WT and mutant worker brains which suggested that mKCs receive both olfactory and visual inputs, mKast may be involved in learning and memory of the multiple sensory information. These are the first functional analyses of honey bee gene of unknown function, paving the way for molecular ethological studies of the honey bee.

Poster Session 2 | Poster Wall 155 | Label: PS2.155

Category: Social behavior and neuromodulation

Implications of nonapeptides in the calling behavior of a South American treefrog

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Hypothalamic nonapeptides (vasopressin-vasotocin (AVT), oxytocin-isotocin) play a crucial role in generating behavioral diversity, fostering temporal behavior patterns that align with the season, immediate social context, and physiological state of the animal. In anurans, the emission of vocal calls in different and complex contexts is an important behavior for successful breeding that can be modulated by AVT. Boana pulchella is a South American hylid frog, widely distributed in Uruguay. During breeding, males congregate in choruses and emit three distinct note types to attract females. In this species, the calling sequences are dynamic and can be modulated by social feedback. Our aim was to investigate how AVT modulates changes in male calling behavior. For this purpose, we conducted pharmacological experiments in the field with three experimental groups of calling males, injecting AVT, AVT antagonist (MC), and saline. We found that the AVT antagonist reduces calling probability and changes the spectral properties of the acoustic signals. Deepening our understanding of how nonapeptides modulate calling behavior appears to be the first step in elucidating the neural mechanisms underlying social interactions in complex contexts.

Poster Session 2 | Poster Wall 156 | Label: PS2.156

Category: Social behavior and neuromodulation

To care or to cannibalize? Machanisms of parental care trade-offs

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Trade-offs between energy acquisition and expenditure intuitively explain many aspects of animal behavior, as individuals must constantly strike a balance between increasing energy reserves through foraging and expending those reserves on interactions with competitors, mates, and offspring. While links at the behavioral level are intuitive and well-characterized, our understanding of how mechanistic connections mediate behavioral trade-offs remains limited. We previously proposed that feeding and social behavior are linked at mechanistic levels, with the modification of feeding related circuits and molecules representing 'variations on a theme' in the evolution and maintenance of social behavior. Here, we leverage the unique biology and rich research history of common coquí (Eleutherodactylus coqui) to test these ideas. Like all animals, coquí must constantly negotiate trade-offs between resource acquisition and expenditure. When the costs of parental investment outweigh the benefits, coquí cannibalize their eggs, an intriguing behavior at the nexus of resource acquisition and social behavior. We first asked how parental state modulated motivation to return home following a disturbance, and asked whether this motivation predicted the propensity to care vs cannibalize. We then compared patterns of neural activity and candidate neuromodulators in the brains of caring vs cannibal frogs. Ultimately, this work will contribute to our understanding of the modification of feeding circuits in the evolution of social behavior and the fundamental question of how evolution builds upon existing substrates to give rise to increasingly complex behavioral systems.

Poster Session 2 | Poster Wall 157 | Label: PS2.157

Category: Social behavior and neuromodulation

Machine learning and mathematical modeling as complementary approaches to understanding marmoset vocal and social behaviors

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Machine learning has emerged as a beneficial tool for ethology, showcasing proficiency in data analysis and pattern recognition. Nonetheless, its interpretability diminishes as models become more biologically realistic. We explore the challenges associated with the interpretability of machine learning models for primate behavior, offering solutions using marmoset vocal and social behaviors as examples. For vocal behaviors, we developed an adaptive boosting-based hierarchical machine learning classifier that accurately determines the caller identity from marmoset vocalizations. It can achieve accuracies of up to 97.8%, surpassing traditional non-hierarchical methods. While it streamlines caller-identity determination and captures inter-individual differences and temporal changes in calls, it falls short in elucidating the mechanisms underlying such changes. To resolve this, inspired by dynamical systems theory, we mathematically modeled the temporal changes in marmoset calls. Our model suggests that marmosets learn certain aspects of calls from their partners, leading to vocal convergence with time, and they do so dynamically while accounting for any changes in their partners' calls. For social behaviors, we used DeepLabCut, a machine-learning-based animal pose estimator, to track points on the body of each marmoset before, during, and after prosocial tasks with another marmoset.
While DeepLabCut's utility was limited to analysis of gaze and hand kinematics, mechanisms underlying coordination could be uncovered by mathematically modeling pairs of marmosets coordinating anti-predator vigilance as coupled oscillators. The model suggested that marmosets estimate the current degree of asynchrony and adjust coordination accordingly. In summary, while machine learning algorithms excel at classification and pattern recognition, mathematical modeling aids in understanding the mechanisms underlying animal behaviors.

Poster Session 2 | Poster Wall 158 | Label: PS2.158

Category: Social behavior and neuromodulation

Seasonality of Neuroestrogens and Their Relationship with Aggression: Insights from the Electric Fish Model Gymnotus omarorum

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Steroids play a crucial role in modulating brain and behavior. While traditionally considered recipients of sex steroids produced in endocrine glands, the brain itself produces steroids. Neurosteroids, synthesized in brain regions regulating social behaviors, play a local regulatory role. While mainly studied in mammals and birds, insights from fish models shed light on neurosteroid synthesis mechanisms and their role on social behavior in vertebrates. Our model species, the weakly electric fish Gymnotus omarorum, is an excellent model to study the seasonality and role of neurosteroids as estrogens affect social behavior differently based on season. In this study, we characterized seasonal changes in estrogen (estrone and 17β-estradiol) levels in plasma and forebrain of wild fish using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Examining the relationship between peripheral and central measurements reveals the brain's sex-specific role in local estrogen synthesis. In males, during both seasons estrogens were detectable in the brain but not in the circulation. In contrast, in breeding females, estrogen levels were similar in the brain and circulation, while in non-breeding females estrogens were detectable only in the brain. These data suggest that neuroestrogens are synthesized year-round in males, whereas in females, they are predominantly synthesized in the non-breeding season. These findings align with previous studies of G. omarorum, indicating that non-breeding aggression relies on estrogen signaling, consistent with observations in bird and mammal models. Overall, our results provide a foundation for understanding the role of neurosteroids, the interplay between central and peripheral steroids, and potential sex differences in regulating social behaviors. Lastly, G. omarorum, is a teleost fish that offers a unique opportunity to identify common strategies in the role of neurosteroids on social behaviors that may have emerged across vertebrates.

Poster Session 2 | Poster Wall 159 | Label: PS2.159

Category: Social behavior and neuromodulation

Chemical cues mediate mound building behavior in termites

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Mound-building termites, Odontotermes obesus collectively construct massive soil-built structures which house their colony. Because worker termites do not possess image-forming eyes which would be required for visual communication, these structures underscore their ability to communicate with each other using chemical signals that are deposited in the mound soil during construction or repair. We developed an experimental assay to assess the ability of termites to distinguish between soils processed by the termites, as compared to fresh unprocessed soil. These experiments reveal that termites use volatile and non-volatile chemicals as communication cues. The presence of these chemicals predisposes the colony members to build at specific sites in a manner that is caste-specific. The non-volatile chemical cues are long lasting, and can elicit construction behaviour for several months. These non-volatile cues appear to encode a colony-specific signature of the mound, allowing termites to distinguish between mounds constructed by members of their own colony from those that belong to other colonies. Moreover, volatile cues combine with non-volatile cues in a manner that elicits different reactions from major vs minor workers. Together, these experiments reveal the complex and subtle chemical communication scheme used by termites during construction.

Poster Session 2 | Poster Wall 160 | Label: PS2.160

Category: Social behavior and neuromodulation

Emerging from solitude: Understanding behavioural transitions of desert locusts through visual and olfactory stimuli

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Desert locusts undergo a drastic phase change, shifting between a solitarious and gregarious form, culminating in population increase and swarming behaviour that pose significant threats to agricultural food supplies. As population density increases, solitarious individuals of the desert locust (Schistocerca gregaria) undergo gregarization, a process marked by diverse behavioural, physiological, and neuronal changes, which we still know little about. Our study delves into the complex dynamics of desert locust behaviour, particularly focusing on the transition from solitarious to gregarious phases and the role of hunger therein. Through behavioural experiments, we investigate how social preferences shift depending on the locust's internal state, shedding light on the classification of solitarious behaviour and the factors driving gregarization. Further, by combining our behavioural assays with functional calcium imaging, we aim to improve our understanding of the neural basis of these behavioural changes. To this end, we investigated how social and food odours are encoded in the locust olfactory system, comparing starved with fed animals. Preliminary findings suggest increased aggregation tendencies with starvation, hinting at a pivotal link between food availability and swarming behaviour.

Poster Session 2 | Poster Wall 161 | Label: PS2.161

Category: Social behavior and neuromodulation

Tethered flight in a robotic tunnel simulator elicits waggle dancing

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Upon their return from valuable resource locations, honeybee foragers perform a stereotypical motion pattern, called waggle dance, which encodes the distance and direction to these resources and eventually recruits new foragers to seek out and find these locations. Foragers estimate the distance they travel via the amount of accumulated optic flow, and their flight direction via their sun compass. Bees flying through a narrow tunnel with patterned walls perceive high optic flow and overestimate the flown distance resulting in waggle dances that advertise virtual, i.e. non-existent, food locations. Here we ask, whether rotating the tunnel changes the dance-reported direction to the food, and, whether the same effect can be achieved by tethering bees in short tunnels with moving walls. Two experiments were conducted, 1) with physical tunnels of up to 20 m length, and 2) using a robotic tunnel simulator with three moving walls (two on the side and one ground panel). Individually marked bees were trained to forage from a feeder placed inside the physical tunnel (1) or inside the robotic tunnel simulator (2). Here, the direction of the wall movement mimicked the in-and outbound flights – interrupted by the feeding period. Waggle dances were video recorded from returning foragers and analyzed after the fact. We find that dances are only performed when foragers can traverse the tunnel with an unobstructed view of the sky and that the dance-indicated direction can be shifted by rotating the tunnel. We find that tethered bees show flight behavior reflecting changes to the direction of optic flow, and consistently perform waggle dances, irrespective of whether they showed flight behavior in the tunnel simulator. This demonstrates the feasibility of our tunnel simulator to control the navigational experience of foragers with applications ranging from research in the neural correlates of navigation and dance communication to controlling recruitment inside a bee colony.

Poster Session 2 | Poster Wall 162 | Label: PS2.162

Category: Social behavior and neuromodulation

Local speed transfers drive the social synchronization of circadian activity in honeybee colonies

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Extensive previous work has investigated the mechanisms behind the synchronization of circadian activity in honeybee colonies, emphasizing the significance of social interactions. However, many prior studies have observed only a few isolated bees in artificial settings, raising questions about the validity of these findings in natural environments. In this study, we used a whole-hive automated detection system to track every bee within the colony throughout their lifespans, analyzing movement data to investigate how social interactions and physical contacts contribute to the spread of circadian patterns within the colony. Agreeing with previous results, we find that rhythmicity and synchronicity increase with age. However, we also detect significant circadian rhythms in the youngest bees and a phase shift in activity from early and old to late and young bees. Combined with the task specificity and spatial fidelity of different age groups in honeybee colonies this results in a wave-like propagation of activity in the hive, spreading from the entrance area and bees displaying intense, synchronized rhythms to younger bees with less pronounced rhythms in the brood area. We find that physical interactions between bees result in speed "transfers", very similar to energy transfers between moving particles. We introduce an agent-based simulation supporting these findings, showing that these speed transfers, combined with circadian variations among hive areas, can account for the observed activity propagation within the colony. Our study provides an intriguing example of how simple rules lead to emergent properties in self-organizing systems, and enhances our understanding of honeybee rhythmicity within complex social structures.

Poster Session 2 | Poster Wall 163 | Label: PS2.163

Category: Social behavior and neuromodulation

Exploring the Determinants of Shoaling Decisions in Zebrafish Using Virtual Reality

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The variability in individual phenotypic traits observed in animal groups is known to modulate social affinity. For example, existing literature in fish suggests that an individual's decision to engage in shoaling can be influenced by its neighbours physical size and speed. At the same time, this influence is likely to be modulated by the individual's own size and/or developmental stage. Yet, the exact impact of such individual traits on shoaling decisions remains elusive due to their strong correlation within natural populations. Immersive virtual reality (VR) offers the opportunity to precisely manipulate features of naturalistic social stimuli, allowing to disentangle specific factors and establish their causal impact on social behavior. By studying zebrafish social interactions with VR, we assess the impact of morphometric and kinematic parameters on shoaling preferences and its variability across different individuals/phenotypes. Our research contributes to elucidating the complex interplay between intrinsic traits and extrinsic social influences that govern shoaling dynamics in fish, with potential implications for understanding mechanisms of group cohesion and fission-fusion dynamics in animal populations.

Poster Session 2 | Poster Wall 164 | Label: PS2.164

Category: Social behavior and neuromodulation

Mapping the brain of the clonal raider ant Ooceraea biroi with transmission electron microscopy

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Ants have been studied extensively due to their advanced social structure and communication strategies, though the neurobiological basis of these behaviors is not well-understood. The clonal raider ant Ooceraea biroi is particularly amenable to investigating the neural circuits underlying social behaviors, as it is uniquely experimentally tractable and genetically accessible. To understand the structural connectivity of the ant brain, we imaged a whole brain at synaptic-level resolution using transmission electron microscopy. This electron microscopy (EM) volume will allow us to reconstruct neurons, identify synapses, and ultimately create a complete wiring diagram, or connectome, of the clonal raider ant brain. To accelerate the mapping of neural circuits in the EM dataset, we used convolutional neural networks to segment neurons and predict synapses. We are now in the process of manual proofreading of these automatic neuron segmentations to reconstruct neural circuits. Finally, to facilitate the matching of cells between EM and light microscopy, we have registered the EM volume to the clonal raider ant reference brain atlas. Given the importance of olfaction in the pheromone-mediated communication of the ant and massive expansion of odorant receptor genes and olfactory glomeruli in the antennal lobe compared to other insects, our initial reconstruction efforts focus on understanding the wiring logic of the ant olfactory system. More broadly, this segmented EM volume, and eventually, complete connectome of the ant brain, adds to the growing toolkit available for investigating the neural circuits that underly behavior in ants.

Poster Session 2 | Poster Wall 165 | Label: PS2.165

Category: Social behavior and neuromodulation

Effect of neuropeptides on the behavioural hierarchy of zebrafish larvae

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Hundreds of neuropeptides appear to be essential in brain function and behaviour, although many remain unstudied. In the zebrafish larvae, the Parathyroid hormone 2 (Pth2) affects social behaviour, corticotropin-releasing hormone (CRH) influences stress responses, and the overexpression of Neuropeptide B (NPB) makes larvae eat more. Yet, it is unknown how broad the behavioural function of these neuropeptides is since they have been tested in few behaviours and show distinct roles in rodents and flies.

In this project, we want to further elucidate the ramifications of neuropeptide roles. Is their influence behaviour and species-specific? Do they have general effects across organisms?

To help explore these possibilities, we created an injection-based, inducible overexpression system to study the effects of these three neuropeptides over nine behaviours of zebrafish larvae. With this system, we can compare the effects over many different behaviours and between orthologues of different species, in a single model. This, along with cutting-edge behaviour-tracking technology, allowed us to understand which behaviours are influenced by each neuropeptide and how specific or general their effects are.

So far, our results show that CRH overexpression increases the probability of escaping from an acoustic stimulus, whereas Pth2 has the opposite effect. NPB overexpression not only makes the larvae eat more, previously reported for other models, but also increases their swimming speed, an effect never described. Experiments with fly and human orthologues of these neuropeptides show similar effects, hinting at conserved functions.

Altogether, this project will contribute to further understand the role of neuropeptides on behaviour within and across species. The use of the method we have developed is helping generalize effects but also find new ones never reported before.

Poster Session 2 | Poster Wall 166 | Label: PS2.166

Category: Social behavior and neuromodulation

Social context of acoustic communication in the small teleost Danionella cerebrum

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The teleost Danionella cerebrum is an emerging model in auditory neuroscience. The males produce sounds in the presence of conspecifics; their small size means they are well suited to ethological studies in the lab, and their transparency allows for optical access to the entire brain. The sounds they produce are composed of pulses, generated at a rate of 60 Hz (‘slow’) or 120 Hz (‘fast’), arranged into discrete bursts that range from a single pulse to hundreds. The bursts are produced in sequences with stereotypical time intervals. We made multi-day recordings of groups of fish in different compositions, including variations in the number of total fish and the sex ratio. Using a hydrophone array, the position of the source of the sound pulse could be triangulated and so, in combination with video tracking, the identity of the vocalising fish could be determined in groups of freely swimming fish. We also conducted replay experiments to study the behaviour of individual fish. Here, we aim to characterise their vocal repertoire and understand the complexity of their vocalisations on a population and individual level, revealing the relationship between the specific burst types they produce and their social context.

We have found that across the population, short fast and short slow bursts are most common. The transitions between similar burst types were more likely than across different burst types, however a first order Markov model was not sufficient to predict the burst sequences. After assigning the vocalisations to individuals, we found in large groups (up to 15 fish) only 3 or 4 fish dominated the sound production. We also observe asymmetries in the order of vocalisations and relative positions of the fish during sound production, implying different roles between fish. By combining our understanding of the vocal structure with the behaviour extracted from the video recordings, we want to build a model that predicts vocalisations from the social context.

Poster Session 2 | Poster Wall 167 | Label: PS2.167

Category: Social behavior and neuromodulation

Neural circuits underlying socially acquired fear memories in mice

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Social learning refers to situations in which individuals learn from others in their social group. Particularly, both non-human animals and humans can acquire fears by witnessing their conspecifics being subjected to adverse events. Experiencing the suffering of others can help observers avoid potential risks, such as predation or poisoning. Brain mechanisms underlying the processing of fear-related stimuli have been mostly studied in non-human animal models where subjects experience fear directly. The study of social transmission of fear in non-human animals from a circuitry perspective that considers the identity of molecules and cells involved is scarce. Oxytocin has been shown to be relevant for several social behaviors, such as consolation, social recognition, among others. Here, we evaluate the role of oxytocin in the formation of social learning in rodents, using the paradigm of observational fear learning in which a subject witnesses a conspecific being trained in a fear conditioning task. Our results suggest that inhibition of oxytocinergic neurons in the PVN negatively Xaffects the ability to form observational fear learning in mice. In addition, we explore brain areas activated after the observational learning task, by studying cFOS activation. We discuss possible circuits involved in the formation of social memories. Studying the mechanisms underlying social fear learning in rodents is key to model and better understand vicarious learning in humans and eventually develop therapeutic strategies for disorders of the central nervous system involving deficits of empathy or social learning such as autism.

Poster Session 2 | Poster Wall 168 | Label: PS2.168

Category: Social behavior and neuromodulation

NEURAL AND MOLECULAR CORRELATES OF VERTEBRATE AFFILIATIVE EVOLUTION

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Animals display remarkable diversity in sociality, provoking fundamental questions about how it has evolved. Although variation in affiliation has independently arisen numerous times across vertebrates, little is known about the underlying neural and molecular mechanisms or how they have evolved. Using immunohistochemical detection of phosphorylated ribosomes (pS6) and oxytocin (OT) cells, we are examining the brain region and molecular correlates of affiliative variation (pair bonded vs. solitary living) within and between species, across five major vertebrate lineages: fishes, amphibians, reptiles, birds, and mammals. We found that a core subset of brain regions, the basolateral amygdala and striatum, are repeatedly linked to social variation across vertebrates, while other brain regions are species- and lineage-specific. Preoptic oxytocin is also repeatedly linked to social variation; however, in a flexible manner across species and lineages. Our findings reveal major neural and molecular themes of affiliative diversity across vertebrates, shedding light onto how it has evolved.

Poster Session 2 | Poster Wall 169 | Label: PS2.169

Category: Social behavior and neuromodulation

Uncovering the mechanisms of testosterone-induced vocal masculinization in African clawed frogs: the roles of perineuronal nets

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Male and female African clawed frogs, X. laevis, emit sexually distinct calls to coordinate reproduction. Both sexes produce calls consisting of a series of clicks repeated at different rates: the male advertisement call comprises clicks repeated at 30 or 70Hz, while the female release calls consist of clicks repeated at approximately 6Hz. Previously, we found that administering testosterone to sexually mature adult female X. laevis resulted in the masculinization of their vocalizations in as little as two weeks, indicating that the central vocal pathways initially producing slow motor rhythms are reconfigured to produce rapid motor rhythms within a short amount of time. Testosterone-induced vocal masculinization thus provides a unique opportunity to explore the neural basis of behavioral modification in adult vertebrates. Perineuronal nets (PNNs) are specialized extracellular matrices in the brain known to inversely correlate with neural plasticity. During development, PNNs progressively increase as neural circuitry becomes more stable. Here, we hypothesized that androgen-induced vocal masculinization is accompanied by a decrease in PNNs in the vocal nuclei that facilitate neural plasticity. Our results, however, showed that the quantity of PNNs in testosterone-treated, fully masculinized female brains was similar to those of sham-operated female brains. We suggest that the loss of PNNs may not be a part of the mechanisms regulating the neural plasticity in the motor systems in adulthood.

Poster Session 2 | Poster Wall 170 | Label: PS2.170

Category: Spatial orientation and navigation

Functional Neuroanatomy of the Goldfish Telencephalon

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Fish, like many other animals, navigate to ensure survival. While the telencephalon region of the fish brain is believed to hold navigation functions, there are contradictory results as to the specific sub-regions in which spatial memory is encoded. Several lesion studies have been conducted trying to determine whether this can be attributed to the lateral pallium or the medial pallium but the results are inconsistent. We aim to answer this question by combining behavioral and lesion studies. Goldfish were trained in three navigation tasks, testing allocentric navigation via horizontal plus-maze, via horizontal bread-board and using hydrostatic cues in a vertical tank. In each task, the fish first learned to navigate to a reward and their success rate was measured. Then, the fish were divided into three groups: lateral pallium lesion, medial pallium lesion, and sham. After undergoing the surgical intervention, the fish success rate in the task was once again measured. Our results indicate that both the medial and lateral pallium are important for the coding of spatial memory, elucidate the function of different regions in the goldfish telencephalon, and contribute to the understanding of navigation in teleost fish in general.

Poster Session 2 | Poster Wall 171 | Label: PS2.171

Category: Spatial orientation and navigation

Celestial Navigation in the Northern Elephant Seal

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Identifying the sensory cues animals use to navigate the ocean remains an open field of research. Despite the scarcity of stable navigational cues in the pelagic environment, marine organisms complete some of the longest known migrations in the world with remarkable precision. For example, northern elephant seals (Mirounga angustirostris) migrate thousands of kilometers from their feeding grounds in the North Pacific Ocean to breed and molt on beaches on the West Coast of North America. It is currently unknown what sensory cues they are using to perform these remarkable navigational feats, but celestial cues and the geomagnetic field are two likely possibilities.

To learn more about the mechanisms behind these animals' movements, we apply machine learning algorithms to biologging data (n = 956,788 positions) from 280 northern elephant seals instrumented with satellite tags. We show that machine learning models purpose-built to handle bio-logging data can accurately predict overhead cloud cover solely from using variables derived from the movement trajectories of the elephant seals. These data suggest the seals may be using a celestial compass to navigate and become disoriented when these cues are not available. Finally, we discuss future applications of these methods to these data to explore whether there is evidence for magnetic navigation in this species.

Poster Session 2 | Poster Wall 172 | Label: PS2.172

Category: Spatial orientation and navigation

Australian Bogong moths use the Earth's magnetic field as a compass for navigation during long-distance migration

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During their annual spring migration, billions of Bogong moths (Agrotis infusa) traverse over 1,000 km to reach a limited number of caves in the Australian Alps, where they undergo aestivation throughout the summer months. Previous research has established that visual and magnetic cues collectively steer the flight behavior of Bogong moths during this extensive migration. However, the extent to which moths rely solely on their magnetic sense to ascertain their inherited direction at night and to navigate towards their destination remains unresolved. In this study, we present evidence indicating that Bogong moths utilize the Earth's magnetic field as a navigational aid during their migratory flight. By tethering moths within an indoor flight arena lined with a panoramic visual scene and surrounded by Helmholtz coils producing a natural geomagnetic field, we observed that moths were oriented in their inherited migratory direction. When the magetic field was turned by 180 degrees, moths oriented about 180 degrees in the opposite direction. In subsequent experiments, when wavelengths of light below 500 nm were obstructed, moths became disoriented. These findings suggest that Bogong moths possess a magnetic compass for long-distance navigation, potentially mediated by cryptochrome. Our study represents the first evidence that nocturnal migratory insects employ the Earth's magnetic field as a compass to navigate.

Poster Session 2 | Poster Wall 173 | Label: PS2.173

Category: Spatial orientation and navigation

Weighting Spatial Memory and Distal Sensory Cues in Egyptian Fruit Bat Navigation

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Egyptian fruit bats use spatial memory for navigation, sometimes prioritizing it over immediate sensory information. Their reliance on spatial memory for navigation can impede their ability to adapt to changes in their environment. This study aims to elucidate the contribution of distal sensory cues to spatial navigation in Egyptian fruit bats. Bats were trained to find a landing perch over repeated trials, invoking stereotyped flight paths. Flight trajectories and echolocation behavior were recorded with high-speed multi-camera video and a microphone array. Movement of the landing perch by 30 cm disrupted the animal's ability to find it, even when visual and echolocation cues were available. The bat's failure to find the new perch location suggested that room landmarks may guide navigation. To determine the influence of distal landmark cues on Egyptian fruit bat navigation, the release and landing locations were rotated in the room by 90, 135 and 180 deg. We posited that if bats rely on distal cues, rotations in the spatial configuration will affect the animal's flight paths. In all rotations, bats consistently missed landing directly on the perch, instead flying in the direction of the new or old perch, or choosing an alternative landing spot. Following 90 deg rotations of release and landing perches, bats visited either the new or previous perch location, but failed to land. In the 180 deg rotation condition, bats flew to the new, rotated perch location, but also failed to land, suggesting they used updated sensory information to navigate toward the perch, but had not yet incorporated distal landmarks into their cognitive map. By contrast, the 135 deg rotation led to disorganized flight trajectories, possibly due to confusion over changes in distal cues. These preliminary findings highlight bats' use of distal sensory cues for orientation, while also showcasing their capacity to adjust navigation trajectories in response to significant environmental changes.

Poster Session 2 | Poster Wall 174 | Label: PS2.174

Category: Spatial orientation and navigation

How dorsal directional and ventrolateral optic flow responses integrate to guide hummingbird hawkmoth flight

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Many animals use visual cues to navigate their environment. To encode their large input ranges, visual systems are adapted to the stimulus statistics experienced in the animals' natural habitats. One strategy to do so is partitioning the visual field; adhering to relevant cues only in areas where they are most abundant or reliable. Such regionalisation can lead to activation of the appropriate visual pathway by target location, rather than by stimulus features. We recently demonstrated this in the hummingbird hawkmoth (Macroglossum stellatarum), which responds with canonical flight adjustments to translational optic-flow exclusively in the ventrolateral visual field, while identical stimuli presented dorsally elicit a novel directional response. Using quantitative imaging of natural visual scenes, we demonstrate that this response split matches the abundance of translational optic flow in the ventral hemisphere, and contrast edges in the dorsal hemisphere (Bigge et al. 2021 Curr Biol. 31:R280-1).

Here we investigated the unique directional flight behaviour of hummingbird hawkmoths to dorsal contrast cues, to test whether it bears any hallmarks of optic-flow-based responses, or even of optic flow avoidance. We analysed the behaviour of hummingbird hawkmoths in flight tunnels and demonstrate that the dorsal directional and ventrolateral optic-flow responses constitute two parallel pathways. Since the hawkmoths' flight responses to dorsal and ventrolateral visual cues are not functionally exclusive, we next analysed their integration hierarchy, using cue conflict experiments in the ventrolateral and dorsal visual field. We propose an integration scheme for the two control systems, and set it into context with the stimulus characteristics of natural visual scenes, to understand the ecological relevance of these flight behaviours for hawkmoth orientation in their natural habitats.

Poster Session 2 | Poster Wall 175 | Label: PS2.175

Category: Spatial orientation and navigation

A robot model of compass cue calibration in the insect brain

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Ball-rolling dung beetles are able to maintain a straight bearing with respect to a plethora of external cues. It has recently been shown that these beetles are able to learn the spatial relationship of available orientation cues, giving them the ability to maintain their bearing even where one of the cues is removed. Behaviourally, this learning is coupled to the dung beetle 'dance', a characteristic rotation performed by the beetles before they roll their dung balls. Mounting evidence in Drosophila indicates that such a learning process is implemented in the insect head direction circuit; specifically, in the plastic substrate between sensory input neurons (ER) and compass neurons (EPG) in the central complex. Critically, this plasticity appears to be driven by rotational movements, providing a clear link with observed beetle behaviour. We recently published a functional model of this circuit and demonstrated that, in simulation, the model could account for beetle cue calibration behaviour. Here, we placed this model on a robot platform and tested it using the same assay as was used for the beetles. The robot performed four exits from a circular arena, aiming to maintain the same bearing each time. The robot started with a single cue (LED sun or wind), performed two exits with a second cue added at 90° (azimuthal offset), then performed a final exit with only the second cue. The plastic connections between ER and EPG neurons should encode the 90° offset between cues, allowing the robot to maintain its bearing on the final exit. The robot was able to replicate beetle behaviour statistically, however the robotic data include significant biasing which is coupled to dance direction. This biasing appears to be caused by inherent conflict between recurrent and instantaneous inputs to the compass circuit. It is possible that the real circuit experiences similar issues or has a mechanism to compensate for the inherent competition between its inputs.

Poster Session 2 | Poster Wall 176 | Label: PS2.176

Category: Spatial orientation and navigation

A Novel Path Integration Circuit in the Ant Brain

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Path integration (PI) is a fundamental navigational strategy used by a number of animals to return to a starting point, such as a nest or shelter, after completing a foraging trip. During an outbound journey, individuals continuously monitor the distances and directions travelled to update their current position with respect to the point of origin. PI is often the primary strategy used by animals to navigate when familiar cues, such as learnt visual landmarks, are unavailable. When familiar cues are available, PI is used as a back-up strategy and often acts as a scaffold for animals to learn visual and other sensory information. Decades of research show that the central complex (CX), a navigational centre in the insect brain, plays a key role in PI. However, the CX remains highly anatomically conserved, even across insects that differ in their reliance on PI. Here, using serial block-face electron microscopy with neuron reconstruction and synapse annotation, we aim to determine the neural circuitry of the CX in three closely related species of ants that have different primary navigational strategies: Saharan desert ant (relies on PI), Australian jack jumper (uses visual landmarks) and Panamanian army ant (uses pheromone trails). We present evidence of remarkable evolutionary stability, particularly in circuits that encode self-motion. When compared to fruit flies, these circuits have remained anatomically conserved over millions of years. Additionally, we discovered a novel circuit that is not present in flies, which we propose could likely provide ants with computational capabilities related to their PI abilities. Species-specific differences in this circuit, in cell number and projection patterns, support the possibility that it may be involved in facilitating diverse PI capabilities. These circuit maps are the first step towards generating a mechanistic understanding of how PI may be encoded in an animal brain.

Poster Session 2 | Poster Wall 177 | Label: PS2.177

Category: Spatial orientation and navigation

Neuroethology of navigation in the real world: Head-direction cells serve as a neural compass in bats navigating outdoors on a remote oceanic island

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Navigation is crucial for animals and humans. Historically, studies of navigation followed two very different approaches: On one hand, ethologists and ecologists tracked animals in the wild, focusing on sensory cues and navigational strategies in real-world environments. On the other hand, neuroscientists recorded neural activity from animals navigating indoors in small laboratory enclosures, and discovered place cells, grid cells, and head-direction cells. However, these neurons were never recorded during real-world navigation, outdoors. To bridge the gap between these two approaches, we conducted the first study of neurons in the brain's "navigation circuit" during outdoors navigation. We focused on head-direction cells, which represent the animal's orientation, and are commonly called "neural compasses". Natural conditions pose several challenges for these neurons to maintain their direction: First, animals move over large geographical spaces. Second, celestial cues such as the moon and sun – the most prominent distal visual cues outdoors – move substantially and disappear completely. However, to this day, no study tested if head-direction cells exist outdoors – and if they do: are they stable over geographical space, and stable to movement of celestial cues – two key properties crucial for any compass. To this end, we developed wireless electrophysiology and high-accuracy positional tracking, and recorded hundreds of single neurons in the presubiculum – a key hub of head-direction cells – while bats were flying and navigating outdoors on a remote oceanic island. We found head-direction cells, which exhibited both key properties of a compass: They showed stable directional tuning over the island's space, and were stable over time – maintaining the same preferred direction regardless of appearance, disappearance, or rotation of the moon. Together, our results suggest that head-direction cells serve as a neural compass during real-world navigation outdoors.

Poster Session 2 | Poster Wall 178 | Label: PS2.178

Category: Spatial orientation and navigation

Fine-scale navigation: how ants perform systematic searches

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Navigation is of crucial importance for the survival of many animal species. To guarantee the successful completion of a journey, insects engage in highly efficient area-restricted searches to finally pinpoint their target. The resulting search patterns are highly structured – systematic – while retaining strong adaptive flexibility. Yet, it remains unclear how this sophisticated behaviour is generated by the insect central nervous system. Our current knowledge suggests that it involves the integration of several distinct navigational steering mechanisms in the insect brain. Here, I investigate the extent to which previous visual experience guides ants during their systematic searches for the nest entrance. When experimentally restricting the area around the nest to prevent foraging ants from forming visual memories, I found that subsequent unrestricted nest searches retained their systematic structure but suffered from very low precision. Asymmetrical restriction led to asymmetrical searches. This indicates that searching ants employ visually guided steering mechanisms, in which the perceived visual environment is matched to visual memories. Where such memories are lacking, the steering mechanism is impeded. Further investigations will explore the contributions of compass-guided and innate steering mechanisms to the systematic search, as well as the underlying neural architecture. As a whole, these findings reveal whether highly sophisticated systematic searching behaviour is indeed generated by the interplay of innate movement routines and navigational modules, and how these routines interact with external cues. More broadly, the outcomes allow us to ask questions of fundamental importance about how brains work to produce adaptive behaviour.

Poster Session 2 | Poster Wall 179 | Label: PS2.179

Category: Spatial orientation and navigation

Time compensation in the celestial compass of insects

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The celestial compass of insects uses skylight to estimate the solar azimuth. However, time-dependent changes in the solar azimuth would cause drifts to the paths of insects that aim to keep a fixed bearing for several hours or revisit their favourite food site after a break. Thus, insects developed a time compensation mechanism to correct their compass for that drift by predicting the changes in the solar azimuth. We propose a computational model of the insects' celestial compass, including a time compensation mechanism, and justify it based on the anatomy of the insect brain. The fan-like arrangement of the dorsal rim ommatidia allows the medulla to directly decode the direction with the highest light intensity or polarisation. The difference between light intensity and polarisation accurately encodes the solar azimuth in a sinusoidal pattern of activity across a population of (MeTu2) neurons. Dorsal neurons provide time information in the AOTu, and their activity follows a sinusoidal pattern across the day-night cycle. We suggest that the sinusoids representing the solar azimuth (MeTu2) and time (DN1pB) combine in the TuBu1 neurons to implement an important trigonometric identity that corrects for the changes in the solar azimuth and result in a geocentric compass. The compass breaks close to the equator, where the changes of the solar azimuth could be either clockwise or counter-clockwise, depending on the time of the year. Thus, migrating insects that cross the equator need a more sophisticated mechanism that requires their latitude on Earth. Interestingly, the latitude is a monotonic function of the magnetic dip, which is detectable by some migrating insects like monarch butterflies. We suggest that the magnetic dip is an input to the celestial compass circuit of some migrating insects, breaking the ambiguity between the northern and southern hemispheres, and effectively transforming the celestial into a true geocentric compass.

Poster Session 2 | Poster Wall 180 | Label: PS2.180

Category: Spatial orientation and navigation

Sparse versus dense coding of very large environments in hippocampal subregions CA3 and CA1

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The hippocampus is comprised of distinct subregions, including areas CA1 and CA3, which differ markedly in their anatomical connectivity. CA1 is largely a feedforward network with almost no intrinsic excitatory connections, whereas CA3 is a recurrent network of densely interconnected pyramidal neurons – which also project to CA1. While the anatomical connectivity differs substantially between these two subregions, the basic firing properties of spatially-modulated place cells in CA1 and CA3 were found by previous studies to be surprisingly similar, consisting mainly of single place-fields with similar field-sizes and spatial information. We hypothesized here that the similar coding properties reported for CA1 and CA3 neurons stem from the small laboratory environment sizes that were used – and that perhaps under more naturalistic spatial scales of hundreds of meters or kilometers the coding schemes of these two areas might differ. In our previously published paper (Eliav*, Maimon* et al., Science 2021) we showed that CA1 place cells recorded in bats flying in a 200-meter long tunnel, exhibit multiple place fields with very different field-sizes for different fields. This multifield multiscale spatial code is fundamentally different from the single fields observed in small laboratory boxes. Here we compared these findings from CA1 to place cells in CA3 that we recorded in bats flying in the same long tunnel. These analyses revealed a dramatic difference between these two subregions: Unlike CA1 place-cells, CA3 place-cells mostly had only single place-fields; however, the sizes of individual place-fields were similar between the two areas. Consequently, the spatial information was significantly higher for place-cells in CA3 versus CA1. These results suggest a fundamental functional difference in neural-coding between these two anatomical subregions of the hippocampus: Sparse coding in CA3 versus dense coding in CA1.

Poster Session 2 | Poster Wall 181 | Label: PS2.181

Category: Spatial orientation and navigation

How do Bees see the World? A normative Deep Reinforcement Learning (DRL) Model for Insect Navigation

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Central place foragers like the honeybee Apis mellifera are masters of visual navigation of the insect world: They can reliably return to their nest under a wide range of visual conditions after a few learning flights. Furthermore, they perform complex navigational tasks, such as 'computing' novel shortcuts between salient locations, supporting claims about the existence of a cognitive map in honeybees. This raises the question which internal representations of the world are necessary and sufficient to explain these behaviors.

We take a normative computational approach based on DRL to address this question, employing a SARSA-like learning algorithm on a single deep neural network to model the processing pipeline from ommatidium-level visual input to an abstract action space in a naturalistic virtual environment. We interpret the output layer as the locus of fast, behavioral learning, operating on complex latent representations of the visual input including e.g. self-motion estimation and memory traces. Anatomically, this output layer maps roughly to dopamine-mediated learning of steering signals to the central complex, based on visual Kenyon cell input into the mushroom bodies. Conversely, shallow and hidden layers represent slow 'learning' (on evolutionary time scales) of visual features and robust, generalizable representations which support rapid, reliable learning of navigational tasks. Within anatomical constraints, we keep the network architecture as general as possible, to maximize the space of possible emerging latent representations and behaviors. This allows us to address questions such as whether path integration and vector memories – essential mechanisms for insect navigation – emerge as a complex representation of visual input from our model and whether Kenyon cells can be considered to have place cell like activity. We further constrain the model by comparing emergent behavior with high resolution observational data over large volumes and timescales.

Poster Session 2 | Poster Wall 182 | Label: PS2.182

Category: Spatial orientation and navigation

Can fish take shortcuts? A study of vector-based navigation in fish

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Navigation is the ability to plan and execute a route to a goal and is key to the survival and success of many animals. One of the strategies used is path integration: a computational ability where individuals continuously update their distance, and angular vector of movement to calculate their position in relation to their departure location. This ability has evolved in both the vertebrate and invertebrate clades and has been shown in mammals, birds and insects. However, evidence is still lacking fish. Yet, determining their ability to path integrate would have implications for understanding both the evolution of navigation and how this behaviour is encoded. With more than 30,000 species, teleost fish are the most successful group of living vertebrates and exhibit remarkable navigation capabilities across a wide range of spatial scales and habitats. Path integration could be a highly advantageous strategy in spatially complex and dynamic aquatic habitats where landmark use can be challenging. Here we tested whether fish can path integrate in an experimental paradigm where navigational parameters and cue availability were tightly controlled. Dominos damsel fish (Dascyllus trimaculatus) were forced to leave their shelter shells in order to reach a food reward chamber in the centre of a circular arena. Once trapped, landmark cues and shelter were removed or shuffled, the fish was released and the trajectories were analysed. Fish exhibit homing behaviour consistent with the use of path integration including error accumulation with the length of the outward trajectory. These results are discussed in an evolutionary and neuro-behavioural framework.

Poster Session 2 | Poster Wall 183 | Label: PS2.183

Category: Spatial orientation and navigation

Simulating insect polarization vision to model a biologically accurate polarization compass

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Animals use external cues to help them navigate in their environments. One such cue used by most insects is the sky polarization pattern, created when the sunlight is scattered in the upper atmosphere, which allows them to estimate the sun's position. Most insects perceive polarized light through a specialized region of their compound eyes called the Dorsal Rim Area (DRA) which exhibits unique properties such as light-scattering pore canals, wide fields of view or, in some species, an extra photoreceptor (1). Although the structural diversity of the DRA among insects has been studied extensively in the past (e.g., (2)), the effects of this diversity on their polarization perception have remained largely unexamined. Furthermore, it is not yet known if the diversification of the DRA (e.g., pore canal variations) could link insects' visual ecologies with the evolution of their polarization vision. Here, I present an insect-inspired and biologically accurate polarization compass that simulates insect polarization vision and sun position estimation through the DRA. Our polarization compass accounts for the physiological features of different insects' DRAs and uses real-life sky data instead of simulated skies to estimate the sun's position in various conditions (e.g. cloud cover). This model expands on previous polarization compass modelling approaches (e.g., (3)) since it allows us to examine the specific effects of divergent DRA morphologies on navigational performances. Finally, our model can serve as an ideal basis for further comparative analyses designed to uncover the functional effect of DRA diversity on insect psychophysics and to unravel the complicated evolutionary history of polarization vision.

1. Labhart T et al., Microsc. Res. Tech., 1999

2. Aepli F et al., Cell Tissue Res., 1985

3. Gkanias E et al., PLoS Comput. Biol., 2019

Poster Session 2 | Poster Wall 184 | Label: PS2.184

Category: Spatial orientation and navigation

Scaling the Leap: Effect of Miniaturisation and Sexual Dimorphism on Jump Kinematics in Australian Spiders

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Spiders of the family Salticidae rely on jumping as their main mode of movement to cross gaps, avoid obstacles, capture prey, and avoid predation. Spiders jump by increasing the hydraulic pressure in their legs which allows them to be extended. They also rely to some extent on leg muscles to power their jumps. Salticids exhibit a 30-fold difference in body weight, with variation seen both between and within species. In addition, spiders jump not only along a horizontal plane, but also jump at different inclinations. Here, we investigated how jump kinematics vary in spiders of different body size. We studied several species of spiders that ranged in size from 5mg to 150mg. In each, we elicited locomotory jumps where animals had to jump a specific distance and different inclinations to reach a visual target. We filmed the jumps using a high-speed camera at 5000fps and recorded 3 consecutive jumps for each individual. We identified the Centre of Mass from µCT scans, tracked this over the jump duration and carried out a frame-by-frame analyses. We report sex specific variation in jump kinematics, with significant differences in acceleration, velocity, kinetic energy and jump force. We also report how these kinematic variables vary along a size gradient. Our results emphasise the need of a comparative approach to study this unique locomotory behaviour.

Poster Session 2 | Poster Wall 185 | Label: PS2.185

Category: Spatial orientation and navigation

The Regulation of Collective Motion in Desert Locusts

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Collective motion, ubiquitous in nature, has traditionally been explained by self-propelled particle models from theoretical physics. Here we demonstrate, via field, lab, and virtual reality experimentation, that classical models of collective behavior cannot account for how collective motion emerges in marching desert locusts, whose swarms impact the livelihood of millions. Challenging previous assumptions, locusts do not explicitly align with neighbors. First, we establish with sensory deprivation experiments in East Africa during a recent major outbreak that this collective behavior relies on vision. While individuals respond to moving dot stimuli via the optomotor response, this innate behavior to wide-field motion does not mediate social response to neighbors. Through observations of locust behavior in immersive virtual reality with holographic conspecifics, arena experiments in which 2000 locusts were tracked, and neural ring attractor models, we show, by contrast, that locust behavior can be explained by a cognitive framework that considers the vectorial representation of neighbors as targets. Our results question long-held beliefs about how order can emerge from disorder in animal collectives and provide a definitive mechanism for locust migrations.

Poster Session 2 | Poster Wall 186 | Label: PS2.186

Category: Spatial orientation and navigation

Propagation and Resolution of Incomplete Information from Polarized Light in Social and Semi-Social Insects

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Socially-acquired information is often incomplete and may be both uncertain and ambiguous. How does this incomplete information affect the individual decisions within the group, and the behaviour of social groups as a whole? It is likely that all insects can perceive and use the polarized light pattern in the sky for navigation. Polarized light is characterized by two properties: the angle of polarization (directional component) and the degree of polarization (signal strength). As uncertainty and ambiguity are inherent properties of polarized light, social animals that use polarized light for navigation, such as locusts and honeybees, are excellent models for investigating how incomplete information propagates through social groups. Locusts are semi-social animals in which communication between individuals occurs passively in the form of inadvertent social cues. The desert locust possess polarization sensitive photoreceptors in the dorsal rim area of their compound eye and it is highly likely that they use a celestial compass system to aid navigation. Although this species has been a model for the neuronal processing of polarized light there is yet scarce behavioural evidence on the orientation of locusts to polarized light, limited mostly to single individuals. Unlike locusts, honeybees are central place foragers that actively communicate the location of a food source to their nest-mates through the waggle dance. Even when presented with uncertain and ambiguous information, e.g. from a polarized light stimulus, a dancing bee will still dance the location to a food source according to her own estimate. However, little is known about how this incomplete information propagates to her nest-mates.

Here, we investigate how locusts and honeybees interpret and make navigational decisions using uncertain and ambiguous information from their peers by altering the directional component, signal strength and the light intensity of an artificial polarized light stimulus.

Poster Session 2 | Poster Wall 187 | Label: PS2.187

Category: Spatial orientation and navigation

Environmental features and intrinsic preferences shape bumblebee goal-learning in virtual reality

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When searching for potential food sources, animals must often navigate complex and cluttered environments. For instance, a foraging bumblebee encounters the challenge of distinguishing rewarding food sources such as flowers amongst numerous other environmental objects that offer no reward. When visiting a flower, the bee must assess its sensory characteristics, nutritional value and location and then decide whether to revisit it on subsequent foraging trips.

Our aim is to uncover the mechanisms underlying this foraging behaviour. Specifically, we want to explore whether bumblebees can learn a visually non-unique goal and distinguish it from other goals of identical appearance but different nutritional value by using visual information from the immediate surroundings. To address this issue, we used a VR-embedded trackball paradigm which allows walking bumblebees to control their visual input as during unrestrained behaviour (closed-loop), but also allows the experimenter to manipulate the visual input without interfering with the animal. Using this technique, we could show that bumblebees can learn to recognise and navigate towards goal locations in VR based on visual cue constellations near the goal and, if they do not get the expected reward, show search behaviour at the presumed goal location. Further, we found innate preferences for certain colours and patterns as well as variations in learning performance depending on the differences between the presented object constellations. These findings shed light on how the intrinsic preferences and learning capabilities of bumblebees shape their foraging behaviour.

Poster Session 2 | Poster Wall 188 | Label: PS2.188

Category: Spatial orientation and navigation

A model and a test of honeybee dance recruit accuracy

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In the waggle dance, a forager communicates the location of valuable resources to nestmates within the hive in the form of a flight vector. Although it has been widely studied, it has remained unclear how nestmates assimilate the information needed to navigate towards the resource. Nestmates following the dance (followers) are required to detect the dancer's orientation relative to gravity and duration of the waggle phase and translate this into their own flight vector with a direction relative to the sun and distance from the hive. In a recent publication [1], we reported a previously unremarked correlation between antennal position and the relative body axes of dancer and follower bees. Based on this, we proposed a plausible neural mechanism that enables followers to assimilate a flight vector that they can follow to the resource. Using real data from tracked dance followers in our model, we obtain appropriately centred but widely distributed estimates of the vector direction. To follow up this result, we devised an experiment to compare the predictions of this model with vectors expressed by real bees recruited to a feeder, as well as experienced foragers returning to a feeder, inspired by the enforced-detour paradigm in ants. Bees were trained to forage from a tunnel at an angle from the hive, and their dances and interactions with nestmates were filmed upon returning to the hive. As a forager or a new recruit began its journey to the feeder, they were caught and forced to fly along a detour tunnel. The angle of the bee's trajectory immediately post-detour was tracked, allowing us to analyse the accuracy of their estimate of the food location from the correction made for the detour. Similar to predictions of the model, we observed a characteristic spread of flight directions centred on the feeder.

[1] Hadjitofi and Webb, Dynamic antennal positioning allows honeybee followers to decode the dance, Current Biology (2024), https://doi.org/10.1016/j.cub.2024.02.045

Poster Session 2 | Poster Wall 189 | Label: PS2.189

Category: Spatial orientation and navigation

Information Flow in Insect Brains at the Synaptic Level

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To study the evolution of neural circuits, we need to know how information flows in the brain at the synaptic level. Serial block-face electron microscopy enables the acquisition of high resolution (~10x10x50 nm3) image volumes of the central complex (CX) – the brain region underlying navigational decisions in arthropods – from multiple species of large insects previously inaccessible for connectomics analyses. Dense annotation of synapses across terabytes of electron microscopy image data would take thousands of hours of manual work, but only tens of hours of computer time if automation is achievable. Building on the work of Buhmann et al. (2021) in the Drosophila melanogaster connectome, we trained a U-Net style convolutional neural net (CNN) to automate the detection of synaptic locations and partners across multiple regions of the CX from a nocturnal sweat bee, Megalopta genalis. This CNN gives us reliable predictions of pre- and post-synaptic pairs in representative volumes of each main computational unit of the CX. Combining synaptic predictions with 3D segmentation of neurons enables reconstruction of complete circuits, with connection weights defined by synapse counts between partner cells. Thus, for the first time, whole brain circuit comparison between mature specimens of multiple species is possible. Additionally, the wealth of synapse data offers the opportunity to extract characteristic visual elements of distinct neurotransmitters. By exploiting a combination of self-supervised and semi-supervised machine learning techniques, we have begun to group neurons by their neurotransmitter identity. Eventually, combining neurotransmitter predictions with detailed information flow data will allow for analysis of neural evolution of CX circuitry across different behaviors, eco-systems, and evolutionary pressures.

Poster Session 2 | Poster Wall 190 | Label: PS2.190

Category: Spatial orientation and navigation

Parallel vector memories in the brain of a bee as a foundation for flexible navigation

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Insects rely on path integration (vector-based navigation) and landmark guidance to perform sophisticated navigational feats, rivaling those seen in mammals. Bees in particular exhibit complex navigation behaviors including creating optimal routes and novel shortcuts between locations, an ability historically indicative of the presence of a cognitive map. A mammalian cognitive map has been widely accepted. However, in insects, the existence of a centralized cognitive map is highly contentious. Using a controlled laboratory assay that condenses foraging behaviors to short distances in walking bumblebees, we reveal that path integration derived home vectors can be transferred to long-term memory, that multiple such home vectors can be stored in parallel, and that these vectors can be recalled at a familiar location and used for homeward navigation. Thus, our results add flexibly recalled memorized home vectors to path integration, memorized food vectors, and view-guided navigation, further increasing the robustness of the navigational toolkit of bees. These findings demonstrate that bees meet the two fundamental requirements of a vector-based analog of a decentralized cognitive-map: Home vectors need to be stored in long-term memory and need to be recalled from remembered locations. Thus, bees possess the foundational elements for a vector-based map. By utilizing this relatively simple strategy for spatial organization, insects could achieve high-level navigation behaviors seen in vertebrates with the limited number of neurons in their brains, circumventing the computational requirements associated with the cognitive maps of mammals.

Poster Session 2 | Poster Wall 191 | Label: PS2.191

Category: Spatial orientation and navigation

An anatomically constrained model for angular velocity integration in the locust brain

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For many navigation tasks, animals rely on an internal compass signal. Our study explores the underlying neural circuitry in the heading circuit of the desert locust Schistocerca gregaria. This ircuit integrates allothetic information such as the position of the sun and idiothetic signals such as rotational self-motion cues to maintain a reliable compass signal.

We propose a novel model for angular velocity integration in an insect heading circuit. Unlike previous models (Turner-Evans et al. 2017 eLife, Pisokas et al. 2020 eLife, Sun et al. 2020 eLife), which feature multiple compass copies and feed-forward mechanisms, our model proposes a single 360° compass signal and angular velocity integration via neuromodulation. Our computational model, based on steady-state firing rate neurons with dynamical synapses, is constrained by anatomical data and optimised using machine learning techniques. The resulting circuit architecture exhibits physiologically plausible neuron activities (Heinze and Homberg 2007 Science, Zittrell et al. 2020 PNAS). Through simulations, we demonstrate the robustness of the neuromodulatory integration mechanism, even in the presence of noise. We suggest that feed-forward and neuromodulatory mechanisms are alternative biological implementations of the shared computational motif of introducing asymmetries to the otherwise symmetric effective circuit connectivity. This motif was first described in a canonical vertebrate heading circuit model (Zhang et al., 1996 J. Neurosci.). Importantly, we show that the heading signal generated by our model can effectively drive goal-directed steering behaviours, suggesting its functional relevance in navigation tasks.

Our study contributes to our understanding of insect navigation but also underscores the complexity and variability of neural systems across species, emphasising the need for nterdisciplinary approaches to unraveling phylogenetically conserved and adaptive neuro- computational principles.

Poster Session 2 | Poster Wall 192 | Label: PS2.192

Category: Spatial orientation and navigation

Modular assembly of the network motifs for vector computation in the insect brain

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The central complex (CX) is a brain region found in the protocerebral midline of all insects. It is characterized by the stereotyped arrangement of conserved neuropil compartments, collections of decussating fiber bundles that interconnect them, and the topographic mapping of neurons comprising these features. This highly regular architecture is crucial to perform the vector computations required to represent and update spatial information during navigation. How are the distinct geometries and precise network motifs of CX elements achieved during development, and what processes may underlie modification of this network during evolutionary change? Insect brains develop from uniquely-identifiable progenitors that divide in a stereotyped manner to give rise to "lineages" of sister neurons. Neurons of a given lineage project along a defined tract, instilling a correspondence between neuron topology and developmental history that can be evaluated at synaptic resolution in connectomic datasets. Here, we segment tracts in the fly connectome, and propose a framework to understand the developmental-synaptic organization of the CX columnar network. In the hemibrain, we identify the four dorsomedial lineages (per hemisphere) that each carve out a quadrant within CX compartments, and posit a non-canonical binary fate choice mechanism by which their tracts segregate. We use these principles to build a developmental simulation that, with minimal tract-based rules, is sufficient to assemble much of the observed network motifs of the fly CX. As future work aims to test simulation generalizability using parameters from other insects, we identified the homologous columnar lineages in the bumblebee projectome and observed a similar developmental organization to the fly. These results suggest a model whereby lineages underlie assembly of the machinery supporting vector computation via an algorithmic process, rather than a complex code specifying every pairwise interaction.

Poster Session 2 | Poster Wall 193 | Label: PS2.193

Category: Spatial orientation and navigation

Flight behavior optimization: Unraveling bumblebee strategies in navigating varied cluttered terrains

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Bumblebees are excellent navigators that can travel long distances on their foraging trips while being able to retrace a path and find back to a known location. Moreover, they do not only forage in open terrains but also in cluttered environments, like forests, where obstacles force the animals to deviate from their paths to avoid collisions.

Becoming an experienced forager and optimizing flight behavior in cluttered terrains is a critical yet underexplored aspect of insect navigation. In this study, we record the flight trajectories of novice bees—those inexperienced in navigating cluttered environments—and monitor their behavioral performance while gaining experience on subsequent foraging trips through numerous obstacles. By carefully controlling for their level of experience, we analyze how flight characteristics evolve with increasing expertise.

Successful navigation in cluttered terrains requires avoiding collisions with surfaces, which can be driven by detecting edges or moving away from visually dark areas. We manipulated the environment by introducing transparent objects and analyzed their impact on the optimization of flight characteristics in different environmental conditions. We find that experienced bees fly similar paths through clutter and quickly learn to adapt their flight, regardless of the environment they were trained in. However, the presence of transparent objects in the environment does influence certain flight characteristics.

In summary, our research provides valuable insights into the adaptive behaviors of bumblebees in cluttered environments, contributing to a deeper understanding of how these insects optimize their foraging efficiency.

Poster Session 2 | Poster Wall 194 | Label: PS2.194

Category: Spatial orientation and navigation

Structure, development and function of the neural circuitry controlling navigation in the Drosophila larva

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Drosophila larvae use olfactory cues to locate food. This process requires the integration of chemosensory information, their innate or learned valence, as well as the mechanosensory (proprioceptive) cues to generate appropriate motor commands. Using this information, larvae navigate up odor gradients by alternating bouts of forward peristalsis ("runs") with stops and turns in the appropriate direction. This behavior likely results from the accumulation of evidence about the direction of the odor gradient across multiple run-casts conducted over the duration of a run (~10 seconds) – a demonstration of short-term memory. Based on our analyses of the larval connectome, we propose that a discrete neuropil compartment in the central brain, the lateral accessory lobe (LAL), forms the core circuit that underlies this navigational feat. Neurons in the larval LAL can be grouped into classes of 2-8 members, based on their developmental origin (lineage) and their projection patterns. Many of these groups engage in dense reciprocal connectivity, forming recurrent networks capable of storing and manipulating information. Specifically in the LAL, we find neuron classes that can (1) integrate olfactory and visual information; (2) combine this information with proprioceptive feedback from chordotonal organs of the body wall. Additionally, downstream, we find recurrently connected excitatory and inhibitory local and commissural neuron classes that are well poised to retain this information in a hemisphere specific manner for timescales that coincide with the run/casts. Finally, the LAL is also the site of origin of a unique population of descending neurons (DNs), which receive directional inputs from all layers of the aforementioned network and project to premotor neurons of the ventral nerve cord. Based on this architecture, we hypothesize that the LAL functions to fine tune turning probabilities of the animal along short time scales thereby sculpting the odor guided searches.

Poster Session 2 | Poster Wall 195 | Label: PS2.195

Category: Spatial orientation and navigation

Compensation to visual impairments and behavioral plasticity in navigating ants

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Desert ants are known to rely heavily on vision while venturing for food and returning to the nest. During these foraging trips, ants memorize and recognize their visual surroundings, which enables them to recapitulate individually learnt routes in a fast and effective manner. The compound eyes are crucial for such visual navigation; however, it remains unclear how information from both eyes are integrated and how ants cope with visual impairment. Here we manipulated the ants' visual system by covering one of the two compound eyes and analyzed their ability to recognize familiar views in various situations. Monocular ants showed an immediate disruption of their ability to recapitulate their familiar route. However, they were able to compensate for the visual impairment in a few hours by restarting a route-learning ontogeny, as naïve ants do. This re-learning process with one eye forms novel memories, without erasing the previous memories acquired with two eyes. Additionally, ants having learnt a route with one eye only are unable to recognize it with two eyes, even though more information is available. Together, this shows that visual memories are encoded and recalled in an egocentric and fundamentally binocular way, where the visual input as a whole must be matched to enable recognition. We show how this kind of visual processing fits with their neural circuitry.

Poster Session 2 | Poster Wall 196 | Label: PS2.196

Category: Spatial orientation and navigation

Developing Parhyale hawaiensis as a comparative model of olfactory navigation

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Odor is an essential cue of food location for animals with diverse brain structures and diverse physical environments. Olfactory navigation behaviors have been well-documented across arthropods (insects and crustaceans), and work in insects has revealed the neural bases of these behaviors. However, little is known about how the crustacean brain, which shares many core structures involved in olfaction and navigation with insects, facilitates olfactory navigation in water. A genetically tractable crustacean model of olfactory navigation would allow us to ask how a basic brain pattern has been adapted to subserve a common behavioral function across different physical environments and body plans.

To address this gap, I am developing a new crustacean model of olfactory navigation in water using the genetically tractable amphipod, Parhyale hawaiensis. I constructed a behavioral imaging apparatus to track locomotion and posture in freely moving Parhyale while foraging for food. During foraging, I find that Parhyale make many brief excursions into the arena center before executing precise ballistic launches towards the food pellet. Close to food, Parhyale exhibit sharp reorienting turns directed towards the pellet. These data suggest that Parhyale employ a sampling and evidence accumulation strategy, rather than gradient search, to locate food. Foraging behaviors are disrupted after selective removal of Parhyale antennules (the homologue of the insect antennae), suggesting that these structures are important for food localization, while removal of the antennae (which are thought to mediate contact chemoreception) disrupts locomotion more generally. Future efforts to adapt neuroanatomically inspired models of insect navigation behavior to explain this behavioral data will allow us to develop hypotheses about how conserved arthropod brain structures have evolved to drive fundamental behaviors across varied environments and body plans.

Poster Session 2 | Poster Wall 197 | Label: PS2.197

Category: Spatial orientation and navigation

Investigating insect spatial learning in virtual reality

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Many insects are known for their ability to successfully navigate between a home location and other behaviorally relevant locations. It is experimentally well established that they learn to identify locations in their environment and revisit these based on memory. At least for flying insects like bumblebees these abilities are mainly dependent on visual cues.

We present a virtual reality (VR) setup which allows the investigation of visual navigation and visual learning mechanisms in insects. By combining a panoramic visual display and an air-cushioned treadmill we created a closed-loop virtual reality in which an insect can walk in a 3D visual environment. We show that in this setup a bumblebee can be trained to visit visually defined locations in a virtual environment when provided with positive and negative feedback in a differential reinforcement scheme. In our experiments, bees consistently learned to visit a rewarded location defined by associated visual objects of different color and/or surface pattern.

By comparing the orientation behavior observed in VR to video-recorded walking behavior of bumblebees in a real-world replicate of an exemplary VR environment, we assessed the quality of immersion of the bees in the virtual feedback loop.

In comparison to real-world experiments targeting the underlying mechanisms of orientation behavior, the controlled manipulation of experimental conditions is much simplified in VR experiments. VR techniques also allow targeted manipulation of the coupling rules in the feedback loop and/or behavior-dependent manipulations of the experimental environment unachievable in physical setups.

Poster Session 2 | Poster Wall 198 | Label: PS2.198

Category: Spatial orientation and navigation

Walking with Dethier: From Local Search Behaviour to Social Communication

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Following sugar intake, hungry flies and active honey bee foragers initiate a local search, where their walking is characterised by increased turning behaviour and path integration-based navigation (Dethier 1957; Brockmann et al. 2018; Shakeel et al. 2023). Interested in understanding how social behaviour evolved, Dethier hypothesised that this solitary behaviour, or parts of it, might have been co-opted in the evolution of honey bee dance behaviour. Building upon Dethier's work, which proposed a link between solitary behaviour in flies and honey bee dance, we conducted a comparative study. We studied the temporal dynamics of local search behaviour in flies and honey bees, and honey bee dance (Shakeel & Brockmann 2023). Through displacement experiments, we found that sugar intake increases the probability to initiate a local search. Interestingly, it is the onset of walking that marks the commencement of path integration-based search. When locomotor behaviour was restricted post-feeding, the heightened motivation for such a search persisted for 3 minutes in both species. Our results suggest that sugar elicits two independent behavioural responses: path integration and increased turning, with the initiation and duration of path integration system being temporally restricted. In contrast, honey bee foragers "delayed" after sugar intake exhibited sustained motivation to initiate dance up to 15 minutes after sugar intake, although the dance intensity declined after 3 minutes. Based on our findings, we propose that in both species, food intake during foraging increases the probability to initiate and modulate locomotor behaviours that can be connected with the path integration system. This ancestral connection may have elaborated during the evolution of honey bee dance to guide the walking pattern.

Poster Session 2 | Poster Wall 199 | Label: PS2.199

Category: Spatial orientation and navigation

neural representation of human experimenters in the bat hippocampus

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Human experimenters are a ubiquitous feature of most laboratory animal studies. Yet, it remains unknown whether the presence and actions of humans influence ongoing neural dynamics of single neurons recorded from subject animals. Neural activity in the hippocampus is known to reflect spatial behavior of the recorded animal and has often been studied with experimenters facilitating the spatial task. Here, we conducted wireless electrophysiological recording of hippocampal neurons from freely flying or stationary Egyptian fruit bats in the presence of human experimenters. In flying bats, we found that a large fraction of neurons modulated their activity depending on the identity of the human at the landing target. In stationary bats, we found that many neurons carried significant spatial information about the position and identity of humans traversing the environment. Combined, our results reveal that hippocampal activity is robustly modulated by the presence, movement, and identity of human experimenters.

Poster Session 2 | Poster Wall 200 | Label: PS2.200

Category: Spatial orientation and navigation

Connectome and functional imaging reveal visual features critical for navigation in Drosophila melanogaster

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The head direction is represented by the activity of compass neurons in the Drosophila central brain, which receives sensory inputs from a diverse set of neurons collectively called the ring (ER) neurons. Many ER neurons are part of the anterior visual pathway (AVP) that conveys visual information from optic lobes to the compass neurons. To investigate how visual information is processed, we used the full adult fly brain EM dataset to densely reconstruct the AVP and trace–at the synapse level–all connections that converge onto each ER population. The spatial connectivity and upstream photoreceptor inputs we uncovered suggest spatially and chromatically distinct processing of visual features by ER types. We tested this prediction physiologically using two-photon calcium imaging combined with a novel projector-based full-color visual stimulation setup. We observed that ER4d neurons responded to vertically elongated visual fields with broad chromatic sensitivity. In contrast, ER2 neurons responded to smaller circular local areas and were inhibited by UV, which was consistent with the model prediction. To further investigate spatial and temporal dynamics of these neurons, we used random-dot stimuli and developed regression models. We found different temporal dynamics of excitatory and inhibitory visual inputs to ER neurons. Interestingly, using the activity of other neurons in the same recording sessions as predictors greatly reduced the total number of predictors in the model, without compromising the model's prediction power, indicating lateral interactions among ER neurons. Overall, ER neurons encode multi-feature visual information and they interact to shape the population activity before being integrated by the head direction system to develop the sense of direction.

Poster Session 2 | Poster Wall 201 | Label: PS2.201

Category: Spatial orientation and navigation

Input from several sources of directional information can decrease orientation precision

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A common notion in cue combination literature is that input from several sources of information facilitates more accurate behavioural output. As it turns out, this is not always the case. In this project, we focused on combining sun and wind to investigate the effect of multiple directional cues on orientation precision. As a model organism we used South African, ball-rolling dung beetles, who to avoid competition for food, shape a piece of dung into a ball and roll it away along a straight path. To maintain its straight trajectory, the beetle makes use of the many orientation cues available to it, including celestial cues and mechanosensory cues. The sun, at a set elevation, intensity, and azimuth serves as a relatively noise-free point-source cue, whereas a wind current has greater inherent noise.

We found that at lower solar elevations, the presence of a sun cue and a wind cue improved orientation accuracy compared to when only one of the two was present. Interestingly, when increasing the solar elevation, orientation accuracy rather decreased in the presence of both cues. It has been shown that when presented with a cue conflict between sun and wind, dung beetles combine cues according to a weighted vector sum and afford greater relative weight to the cue that is the most reliable at any moment in time (Shaverdian, Dirlik, Mitchell et al., 2022). The behavioural results in the present study suggest that the influence of cue noise on the behavioural output is dependent on cue weight; as the relative weight of the directional cues shifted towards the wind, its inherent noise began to weigh more as well. A recently proposed neural model of cue integration in the dung beetle brain, that combines cues according to a vector sum, yields comparable results (Mitchell, Shaverdian et al., 2023). Together, these findings clearly demonstrate that having input from several sources of information does not necessarily generate the most accurate behaviour.

Poster Session 2 | Poster Wall 202 | Label: PS2.202

Category: Spatial orientation and navigation

Knowing your neighbourhood: How cluttered environments shape initial learning flights of bumblebees

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It is crucial for central place foragers, navigating between their home and food sources, to learn the surroundings of their home. Bumblebee nests are often concealed underground with inconspicuous entrances and tend to be situated amidst cluttered environments such as meadows or areas near bushes and trees. Upon leaving their nest for the first time, bees exhibit convoluted movement patterns, increasing their distance and altitude while frequently looking back to the nest entrance. Returning home to their nest requires this periodic “turn-back and look” behaviour to visually memorise their environment. These memories (often called snapshots) are thought to be utilised during their return journey to orient themselves in the familiar direction.

While several studies have revealed great details on the learning routine of bees when leaving their nest, they used only a few conspicuous landmarks and did not assess the role of the immediate environments on the learning routines. Here, we investigated in a cylindrical flight arena how the initial outbound flights of bumblebees are shaped by the number of objects and the density of their arrangement cluttering the nest surroundings. We found that bees gain altitude faster when surrounded by a dense object arrangement compared to scenarios with fewer objects in proximity to the nest entrance. Furthermore, we explore how these surrounding objects affect the “turn-back and look” behaviour. It is yet unknown whether surrounding objects solely obstruct or potentially create an additional attraction beyond the nest position. These insights help in understanding how environmental features influence navigation and the learning processes in pollinators.

Poster Session 2 | Poster Wall 203 | Label: PS2.203

Category: Spatial orientation and navigation

Maintaining head direction estimates in natural scenes with a spiking neural network model of the central complex and active behavioural strategies

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Maintaining a stable estimate of heading direction is an essential component of many behaviours across species. In insects, heading direction is tracked by ‘compass neurons’ in the central complex which show activity with strong directional tuning. This estimate of heading direction can be maintained with angular self-motion information (e.g. from efference copy and/or optic flow), but is subject to accumulating error making the estimate increasingly inaccurate. To mitigate this error, heading direction is additionally stabilised by external visual features. Specifically, the insect learns the relationships between visual information arriving from visual ring neurons and the compass neurons representing its current heading, such that when a visual scene is revisited the appropriate heading is recalled despite noisy self-motion cues.

Using a spiking neural network model of the insect central complex, we first show that simple visual filtering by ring neurons with Drosophila-inspired receptive fields is sufficient to form a mapping between heading and complex natural scenes. This mapping can maintain the heading estimate in the absence of self-motion information. However over extended periods, visual features will change and ring neuron to compass neuron mappings will become inaccurate. We show that using active behavioural strategies to resample headings can support the maintenance of accurate heading estimations. As natural intelligence is a property of closed loop brain-body-environment interactions, we test this on board a small robot, navigating over extended times and distances in dynamic environments.

Poster Session 2 | Poster Wall 204 | Label: PS2.204

Category: Spatial orientation and navigation

Identifying neuromodulatory input neurons to the fan-shaped body of the insect central complex

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Insects exhibit exceptional navigational abilities utilizing a compartment of their brain known as the central complex (CX). The CX comprises four distinct neuropils. These include the protocerebral bridge (PB), the fan-shaped body (FB), the ellipsoid body (EB) and the noduli (NO). Functionally, the CX has been implicated in encoding the insect's current heading, its goal heading, and in instructing the insect's steering decisions if these two angles do not match. Which goal heading is appropriate in any given situation depends on internal and external context, likely mediated via neuromodulatory input to the FB. The integration of directional information and contextual information in the FB thus forms the basis for context dependent action selection, providing different species with their unique behavioral repertoire. To reveal the concrete neural substrate of neuromodulatory input to the FB, we have performed volume electron microscopy based neural reconstructions of FB tangential neurons as well as immunohistochemical labeling of neuromodulatory transmitters. These include serotonin (5-HT), dopamine (labeling of tyrosin hydroxilase), as well as several neuropepides. Labeling for these neuromodulators was confined to a limited set of tangential neurons in distinct FB layers, allowing 3D tracing of their main branches. These were then matched with single neuron tracings resulting from our connectomics data to obtain likely neural identities of modulator expressing neurons. Finally, the placement of these cells relative to other neurons of the FB circuitry was analyzed, with particular focus on connections to the main output neurons of the CX, i.e. the likely site of integration of contextual with directional information. Overall, our results are suited to shed light on the evolution of the neural circuits in the FB, illuminating conserved core circuit motifs as well as circuit aspects possibly involved in mediating species identity.

Poster Session 2 | Poster Wall 205 | Label: PS2.205

Category: Spatial orientation and navigation

Studying 3D navigation in the spontaneously behaving common marmoset

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During their daily activities, non-human primates need to orient in their environment in order to locate themselves, remember relevant sites, and plan routes. Common marmosets (Callithrix jacchus) are New World monkeys, equipped with accurate foveal vision similar to humans, and renowned for their exceptional climbing ability. Therefore, they are an ideal and human-relevant model for investigating navigation in intricate settings.

We designed and built multiple setups (up to 4m3) to study navigation in freely behaving marmosets. We use a marker-based motion capture system (Optitrack; 24 cameras) to robustly track multiple animals' head movements simultaneously in 3D, and we plan to use data-loggers (White Matter) to record up to 64 channels of neuronal data.

We are interested in the head direction cells coding of the 3D space which until now has been studied in freely moving rodents or in animals passively being rotated. Thanks to their climbing proficiency, marmosets are an interesting model to study 3D orientation. Accordingly, we easily trained them to locomote for several minutes on the floor and ceiling of a cage, and demonstrate that the resulting head motion efficiently covers 3D rotation space. The further use of this setup will lay the groundwork for future research in the field of 3D orientation.

Poster Session 2 | Poster Wall 206 | Label: PS2.206

Category: Spatial orientation and navigation

Neuroanatomical variations in the ball-rolling beetle's central complex

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The ball-rolling beetle Kheper lamarcki is one of many dung beetle species found at a dung pile. To avoid competition for food, the ball-rolling beetle demonstrates an evasive behaviour where it forms a dung ball and rolls it away along a straight line. This orientation strategy is one of many adopted by insects. The neural computations underlying insect orientation take place in a central, well-conserved region of the insect brain termed the central complex (CX). The CX has been identified in the ball-rolling beetle, although many details have yet to be described which raises the following question: How does the CX circuitry of the brain of the dung beetle compare to that of other insects?

A novel microscopy technique, serial block-face electron microscopy, permitted us to image the entire CX in the ball-rolling beetle. Within the CX, we further focused on two circuits known previously to have variation in their neuron projections and to be essential for orientation in insects. 1) In the insect head direction circuit, we found a completely novel projection pattern. We hypothesize that this alternative projection pattern still allows the beetle to accurately track its head direction when completing a full 360° turn. 2) In the second circuit, computing self-motion, we found the same number of self-motion neurons as in the bumblebee but an overall layered organisation like in the fruit fly. We hypothesize that these novel projection pattern still relay the same five modalities of self-motion into the CX as observed in other insects.

In conclusion, we suggest that despite the described neuron projections differences, the functionally of the beetle circuits still overlaps with that of other insects to support orientation.

Poster Session 2 | Poster Wall 207 | Label: PS2.207

Category: Spatial orientation and navigation

Integration of navigational cues in the central brain of Drosophila melanogaster

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Drosophila melanogaster serves as an excellent model organism for dissecting the intricate neural circuitry underlying specific behaviors in the brain. With approximately 200,000 neurons comprising its brain, the fruit fly demonstrates considerable neuronal complexity, while also manifesting remarkable capabilities in executing diverse behavioral tasks. Central to this proficiency is a sophisticated visual system enabling, for instance, the discrimination of colors or the detection of motion, leading to its ability to both differentiate between various visual cues, as well as to integrate them. Particularly noteworthy is the fly's ability to navigate through space, while utilizing different cues such as the sun's position, chromatic gradients, or polarized skylight as a reference. These cues are thought to be processed along an evolutionarily conserved circuit known as the anterior visual pathway (AVP) across insects.

We focus on two distinct AVP pathways implicated in the processing of polarized skylight, seeking to understand their contribution to informing navigation: (1) TuBu cells, connecting the anterior optic tubercle (AOTU) to the central complex (CX), and (2) TuTu cells, connecting the AOTUs of both hemispheres. We use a combination of anatomical methods (immunohistochemistry and electron microscopy-based connectomics) in combination with both genetically encoded indicators of neuronal activity (Calcium imaging) and molecular genetic manipulations of behavior in order to ascertain whether these cells are related to specific behavioral phenotypes (using virtual flight arenas).

Overall, this interdisciplinary approach integrates molecular, cellular, and behavioral methods to dissect navigational circuitry in Drosophila. The findings from this study promise to provide valuable insights into the fundamental principles underlying sensory-guided navigation in insects, with potential implications for understanding similar processes across diverse species.

Poster Session 2 | Poster Wall 208 | Label: PS2.208

Category: Spatial orientation and navigation

The importance of dorsal landmarks for navigation in dark and cluttered forest environments

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Many insects rely on visual landmarks to localize their 'home' or to return to a food source. Much of our knowledge about landmark navigation comes from work on a few species that inhabit 'open' visual environments, for example the desert ant, Cataglyphis bicolor. This work stresses the importance of landmarks around the horizon for navigation in these species, which makes sense given the lack of overhead or dorsal landmarks in open environments.

However, the usefulness of landmarks in different regions of the insect visual field likely depends on the visual environment. In a cluttered jungle for example, the patches of sky visible through the canopy creates a pattern of landmarks in the dorsal visual field which could provide guidance over similar distances to landmarks on or below the horizon (e.g. tree trunks and leaf litter). Additionally, these canopy-pattern landmarks have higher contrast than laterally placed landmarks, particularly in dim light, further increasing their usefulness, especially for animals navigating in forest environments at night. In contrast, in an open landscape like a desert, frontal landmarks are visible over vast distances, and dorsal landmarks usually do not exist.

In this poster I present behavioural experiments conducted on the partly forest-dwelling wasp, Vespula vulgaris, to test whether they can use both artificial landmarks and naturalistic 'canopies' within their dorsal visual field to locate a food reward. I also present plans for future experiments.

Poster Session 2 | Poster Wall 209 | Label: PS2.209

Category: Spatial orientation and navigation

Distinct navigational strategies underlie orienting behavior in fruit flies

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Many animals, including insects, are thought to use head direction (HD) estimates derived from global visual cues to navigate. When and how HD estimates drive moment-to-moment navigational decisions remains unclear. In flies, HD cells in the central complex — a highly conserved brain region in insects — are important in selecting and maintaining individualized heading preferences relative to prominent visual and mechanosensory cues in the environment. Here, we used virtual reality (VR) setup compatible with 2-photon imaging to study the behavior of tethered walking flies and their HD estimate in diverse visual scenes that included celestial cues in isolation or in combination.

1. We confirmed that wild-type flies exhibit individualized heading preferences in the presence of directional cues that mimicked celestial objects such as bright sun-like disks and intensity gradients.

2. Flies' ability to orient reliably was affected by the contrast of the directional cue but unaffected by the addition of a distinct secondary cue presented either in combination or in conflict.

3. Even though flies maintained their individualized heading preference on average over several minutes, they showed a tendency to switch to (negative) phototaxis — a behavior in which they oriented away from bright cues.

4. The tendency to switch, as well as the stability of the HD estimate, were history dependent and affected by the visual contrast of the directional cue. When we silenced HD cells in the central complex, all flies switched to phototaxis.

Our results demonstrate that flies with an intact HD system can switch between apparently distinct navigational strategies depending on their history and visual environment.

Poster Session 2 | Poster Wall 210 | Label: PS2.210

Category: Spatial orientation and navigation

Dialects in honey bee dance communication

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The hypothesis that populations and species of honey bees vary in dance communication of flight distance goes back to Karl von Frisch (1948). He called the phenomenon dance dialect linking it to regional differences in human and bird vocal communication. Recent common garden experiments with several honey bee species and geographic populations of Apis cerana conclusively confirmed the existence of variation in distance communication (Kohl et al. 2020, Bharath Kumar et al. 2024). Species and populations differ in the resolution with which they indicate differences in distances, experimentally determined as differences in the slope correlating dance duration and flight distance. Fred Dyer (1991) proposed that limitations in the signal production or reception impede the optimization of the communication process, and that populations or species with large foraging ranges trade off spatial resolution for maximal distance communication. There is some evidence supporting a negative correlation between foraging range and steepness of dance calibration curves, but thorough studies are missing. Now, we propose that there are two equally plausible causes for the tradeoff of spatial resolution: (i) limitations in the communication processes as Dyer (1991) suggested, and (ii) limitations in the representation of spatial distance. In the latter case, differences in the slopes of dance calibration curves would indicate differences in the representation of space instead of differences in the translation of flight distances into dance movements. Currently, there is not much known about the representation of spatial distances in insect brains and differences in distance coding between small- and large-scale insect navigators. We suggest that the honey bee waggle dance could be a fruitful behavioral paradigm to explore neural mechanisms of distance coding in insects. The poster will present a review of the available data and a discussion of possible research strategies.

Poster Session 2 | Poster Wall 211 | Label: PS2.211

Category: Spatial orientation and navigation

Navigation and orientation in Drosophila melanogaster – Do larvae keep track of there spartial position?

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Orientation and navigation within a changing environment are crucial for an animal's survival. External cues such as odors or visual landmarks enable animals to find food sources, mating partners or predator-free places. Even in the absence of this allothetic cues animals can perform a goal-directed navigation by using idiothetic information. This navigational strategy is based on an internal presentation of the environment and error-prone self-motion cues which are integrated, processed, and represented within the central complex. Previous studies in adult Drosophila melanogaster revealed that flies use idiothetic cues to search locally for a previous food source. In this study we observed a similar behavior in Drosophila melanogaster larvae. Due to the establishment of the larval local search paradigm combined with the tracking of the larval movements via FIM, we identified parameters describing the larval local search. After the interaction with a chemical stimulus-filled container, larvae performed a centralized search that is influenced by external and internal conditions. Through the involvement of several control groups, we identified that the local search is neither the result of potentially remaining chemical cues nor time. Since larvae have not a functional central complex the nature of this behavior remains unknown and will be the subject of future studies.

Poster Session 2 | Poster Wall 212 | Label: PS2.212

Category: Spatial orientation and navigation

A model for visual decision making and exploration in Drosophila melanogaster

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In resource-rich environments, flies fly straight paths interspersed with rapid turns of approximately 90 degrees, known as saccades. Saccadic behavior increases the area covered during exploratory flight while optimizing the chances of localizing resources. Despite decades of research into saccade behavior in flies, neither the exact environmental stimuli nor the neuronal computations leading to a saccade are well-understood

Using a flight simulator, we investigated how flies of the genus Drosophila melanogaster explore naturalistic 3D virtual landscapes. Repeated testing of flies revealed inter-individual variability in saccade properties that remain stable over consecutive days, leading to diverse yet individually consistent exploratory phenotypes within a fly population. An analysis of changes in the visual field prior to saccades revealed individual spatiotemporal saccade "trigger" sensitivities that influence individual inter-saccade intervals and saccade direction, giving rise to a variety of individual exploratory phenotypes.

Testing flies in realistic (based on high-resolution 3D satellite data) and unrealistic (modifications) virtual environments allowed for computing a predictive model of 12 visual parameters and their influence on saccade direction, which can explain the directionality of two-thirds of all turns. Subsequent testing of our model using simulated flies flying in the same virtual reality demonstrated that effective exploration of unknown terrain requires stochastic decisions to prevent the flies from being overly fixated towards points of high visual preference, which would result in low population dispersal.

Our current efforts concentrate on investigating the neuronal underpinnings of saccade computations.

Poster Session 2 | Poster Wall 213 | Label: PS2.213

Category: Spatial orientation and navigation

Fast positional reference formation in hover feeding hawkmoths

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Animals orient themselves in space to perform a number of behaviors, including central-place foraging, menotaxis, landmark-based navigation, target-tracking and prey-capture. Some of these directed behaviors may require an explicit positional reference in space, while others may use purely directional cues. These different neuronal mechanisms can exhibit similar movement outcomes. In transient behaviors, a reference point may be learnt and reset quickly, making its identification a challenge.

We studied flower-tracking and hover-feeding behavior in the hawkmoth, Manduca sexta. Using high-speed cameras and robotic flowers, we measured the dynamics of flower-tracking. We observed that during each floral encounter, individual moths maintained a fixed offset relative to the flowers before, during, and after a bout of flower motion. This offset persisted over a range of flower motion speeds. Using time-series analysis, we showed that this offset is observed over tens of seconds, with mean-reverting properties. This indicates the presence of a persistent, multisensory reference-point in flower-tracking, which we call position-locking.

In addition to persistence, the reference-point is reformable. Individual moths feeding more than once, as well as different individuals, showed position-locking at different points, usually wherever they first made contact with the nectary and got a sucrose reward. Thus, the positional reference is re-formed at the start of each feeding bout.

These observations may be explained by either an explicit positional representation, which would be a form of positional memory, or a re-zeroing of a velocity integrator. Using control-theoretic models of mechanosensory and visual flower-tracking, we predicted the system's response to visual jumps, as an additional test of the underlying reference-forming mechanism. Taken together, these results show a fast, reward-gated form of reference-point resetting, inferred from timeseries analysis of behavior.

Poster Session 2 | Poster Wall 214 | Label: PS2.214

Category: Spatial orientation and navigation

Altitude-dependent perception of ambient light conditions

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One of the most dramatic changes occurring on our planet in recent decades is the ever-increasing extensive use of artificial light at night, which drastically altered the environment nocturnal animals are adapted to. Unlike animals moving exclusively on the ground, flying animals can move vertically in addition. One parameter considerably changing with altitude is the visual range and thus the light environment perceived by an individual. To demonstrate this, we performed measurements at the Watzmann, the central massif of the Berchtesgadener Alps. At this special location, it was possible to use ground-based all-sky photometry at different altitudes due to the steep incline, demonstrating the crucial differences depending on altitude. Moreover, our recent findings revealed by harmonic radar indicate that flight altitude is decisive for the attraction of moths towards an artificial light source. We therefore propose to extend the attraction radius of a light source, which is conceived only in two dimensions to date, by the third dimension. Since the disturbance of natural light patterns by artificial light is altitude-dependent, we emphasize the need to include altitude as a vital parameter in the study of ecological light pollution and encourage further research to determine its role as a predictor of light-related effects on airborne organisms.

Poster Session 2 | Poster Wall 215 | Label: PS2.215

Category: Spatial orientation and navigation

Unravelling the effects of rotations on oriented behaviour

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Every day in the South African savannah, millions of dung beetles from near and far follow the smell of animals' fresh waste products. At the dung pat, each beetle forms a dung ball, and escapes the competition for food by rolling it away in a straight line, before consuming it in peace. This straight-line orientation leads it as far away from the competition as fast as possible. At the start of their journey, the beetle climbs on top of the ball and performs a series of rotations around its own body axis: the orientation dance.

The ball-rolling beetles perform their orientation dance to create snapshots of available directional cues. They may also repeat this behaviour along their path to regain their initial bearing after experiencing disturbances. Interestingly, ants also perform saccades while learning the surroundings of their nests before foraging, and sandhoppers use body rotations for detecting the Earth's magnetic field. As a matter of fact, rotations are even necessary to learn an internal representation of external directional cues in the brain of fruit flies.

While studying navigating dung beetles in the field and in an indoor arena, we characterised the components of the orientation dance and its effects on orientation behaviour. We find that beetles followed available directional cues more precisely the more they danced. Surprisingly, our results suggest that dancing may increase the overall tortuosity of a beetle's path, thus hindering its progress under some conditions. In summary, our findings consolidate the orientation dance as an essential component of straight-line orientation and navigational cue integration in ball-rolling dung beetles.

Poster Session 2 | Poster Wall 216 | Label: PS2.216

Category: Spatial orientation and navigation

A novel navigation circuit in the hymenopteran central complex

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At the intersection between sensory and motor pathways, the central complex (CX) is a region of the insect brain involved in flexible decision-making during navigation. Extensive research in Drosophila melanogaster, including a connectome of the CX, notably support its role in vector navigation. However, neural circuits and behavioral repertoire of the fruit fly might differ from other insects, notably those capable of more complex navigation behaviors.

To fill this gap, we used block-face electron microscopy to acquire multi-resolution image stacks of the CX of three hymenopteran species: bumblebee, honeybee, and a sweat bee. We generated projectomics and connectomics data using manual tracing in all three bees, and automatic image segmentation and synapse detection in the sweat bee.

We focused on PFN neurons, known to play a key role in vector navigation. Despite large differences in neuropil layout and more than twice the number of PFN cells in bees, we identified analogous circuitry resembling that of the fly, suggesting that it is conserved across species. Interestingly, we revealed a new PFN subtype in the bee CX (PFNc). These PFNc cells account for circa half of all PFN neurons and project to a compartment of the nodulus (NO) only found in hymenopteran insects, the cap unit (NOc). They connect to bee-specific FBt-NOc, tangential cells projecting from the superior neuropils (SNP) to the fan-shaped body (FB) and the NOc.

In the sweat bee, connectivity analysis showed that the novel PFNc neurons and FBt-NOc cells form several parallel, recurrent circuits between the NOc and the FB. Although their function is unknown, FBt-NOc neurons are reminiscent of a putative neuron type hypothesized to encode long-term vector memory in a model suited to explain complex vector navigation (Le Moël et al. 2019). We suggest that FBt-NOc cells could receive valence information from the mushroom bodies via the SNP, providing the foundation for species-specific complex navigation.

Poster Session 2 | Poster Wall 217 | Label: PS2.217

Category: Spatial orientation and navigation

A projector-based virtual reality display for 3D insect flight

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When navigating familiar environments, bumblebees robustly and efficiently locate known food sources and nest sites by using visual memories of previously-encountered landmarks. The neural circuits underlying this feat have not been experimentally confirmed. To facilitate neural recordings from the bumblebee brain during naturalistic, visually-guided navigation, we developed a virtual reality display tailored to tethered, closed-loop 3D insect flight. Current VR displays suffer from design trade-offs leading to either higher cost, space-constrained system integration, longer latencies, lower resolution or lack of full coverage of insects' spectral sensitivities and field of view. Our projector-based display largely addresses these limitations. By utilizing five low-cost (0.4 MP) projectors and a rear-projected dome adapted to the geometry of other experimental equipment, the animal's field of view can be covered nearly completely. A 240 Hz frame rate ensures low-latency visual feedback during closed-loop flight. The illumination was chosen to match the green, blue and ultraviolet spectral sensitivities of bumblebees, typical also for many other insect species. To facilitate naturalistic interaction with food sources, we designed a movable walking treadmill to allow takeoffs and landings. While initially our assay utilizes a force sensor to measure turning and forward body accelerations, we aim to develop a vision-based 6-degree of freedom body acceleration sensor. Once implemented, this system is suited to facilitate more naturalistic 3D kinematics during tethered virtual flight. Overall, our display system combined with the anticipated force sensing will open the path towards elucidating the neural underpinnings of memory-guided decision-making during naturalistic foraging in bumblebees and other flying insects.

Poster Session 2 | Poster Wall 218 | Label: PS2.218

Category: Spatial orientation and navigation

Investigating visual navigation with spiking neural network models of the insect mushroom bodies

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Ants are amazing visual navigators, capable of learning long visually guided routes with limited neural resource. This visual memory is mediated by the mushroom bodies; an insect brain region important for learning and memory. In a visual navigation context, the mushroom bodies are theorised to act as familiarity detectors, guiding ants to views that are similar to those previously learned when travelling along a route. Evidence from behavioural experiments, computational studies and brain lesion studies all support this idea. Here we further investigate the role of mushroom bodies in visual navigation with a spiking neural network (SNN) model learning complex natural scenes. We implemented these networks in GeNN (a library for GPU accelerated SNNs) allowing us to test models offline on an image database of scenes from a complex natural environment, and also online embodied on a robot. The mushroom body model successfully learnt a large series of visual scenes (400 scenes corresponding to a 27m route) and used these memories to choose heading directions during route recapitulation. We demonstrated that KC activity is directly related to the respective novelty of input images. Through conducting a parameter search we found that there is a non-linear dependence between optimal KC to visual projection neuron (VPN) connection sparsity and the amount of stimulus presentation time. The parameter search also showed training on lower proportions of a route generally produced better navigation accuracy. We embodied the mushroom body model and comparator visual navigation algorithms on a Quanser Q-car robot with all processing running on an Nvidia Jetson TX2. On a 6.5 m route, the mushroom body model's navigation accuracy was comparable to standard visual-only navigation algorithms. Thus, we have demonstrated that a biologically plausible model of the ant mushroom body can navigate complex environments both in simulation and the real world.

Poster Session 2 | Poster Wall 219 | Label: PS2.219

Category: Spatial orientation and navigation

Navigation with touch

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Naked mole-rats (Heterocephalus glaber) are one of the most incredible creatures in the animal kingdom. They are a unique eusocial rodent species with extraordinary longevity(1) and able to communicate with each other with versatile colony-specific dialects(2) and can even survive under extreme hypoxia by switching to fructose as a fuel(3). Their subterranean burrows are large and complex with multiple branching tunnels, which can spread more than 3km, but mole-rats orientate themselves and navigate without reliance on visual cues(4). However, the underlying mechanisms used by these animals to navigate have not yet been studied. Our results suggest that naked mole-rats utilize their densely innervated body hairs as tactile navigational tools. Behavioral experiment indicates that the deflection of these hairs as they move down narrow tunnels aids in accurate navigation. Additionally, mechanoreceptors innervating these hairs are tuned to certain deflection angles, probably providing directional information feedback. Moreover, these receptors can also fire continuously for minutes in response to static displacement, potentially providing information on time travelled as the hairs remain bent by tunnel walls during movement. Collectively, our findings illuminate the sophisticated tactile-based navigation system employed by naked mole-rats.

Poster Session 2 | Poster Wall 220 | Label: PS2.220

Category: Spatial orientation and navigation

Distance dependency of light attraction in flying insects

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Artificial light can dramatically entrap night-flying insects by corrupting their dorsal light response and by leading to unstable flight modes like looping, stalling and inverting. However, not all insects may react in the same manner and how this behavior changes further away from a light source has been an open question. We present findings from field data analyzing 3D flight patterns of different insects flying to light filmed at different distances. We find that a range of insect taxa (9 orders) including flies, wasps, moths, beetles, bugs, termites and katydids show similar light entrapment effects at night with some differences in frequency of motifs across different orders and showcase 3D flight paths for these different taxa. We find that unstable modes of flight predominantly occur within each order in the presence of upwelling or downwelling point sources and extended tube sources, but dramatically reduce with diffuse downwelling light, reaching similar frequencies as complete darkness. We also compare flight path metrics like tortuosity, speed and as a function of distance from light to see if this approach can yield some estimates of how far from a light these unstable modes or other behaviours leading to light entrapment start to appear.

Poster Session 2 | Poster Wall 221 | Label: PS2.221

Category: Spatial orientation and navigation

Feeding site fidelity in Mauritian flying foxes Pteropus niger

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Imagine you are going out and need to choose a restaurant. How do you choose where to dine today? Do you go to the last place you visited, use a friend's recommendation or just wander the streets until you smell something delicious?

Foraging frugivorous bats encounter these problems every day, while navigating through a lot of additional factors such as food availability, seasonality, and competition. This research explores the mechanisms behind the feeding site selection process in Mauritian flying foxes. Utilizing ATLAS (a reverse GPS-system), we tracked the movements of over a hundred Pteropus niger bats endemic to Mauritius over a period of up to seven months. Prior to tagging, each bat underwent a series of personality assessment experiments. Through our presentation, we will demonstrate the preliminary conclusions on how the factors such as sex, reproductive state, and personality traits influence feeding site fidelity.

Poster Session 2 | Poster Wall 222 | Label: PS2.222

Category: Vision and photoreception

Pretectal neurons in zebra finches modulate with optic flow during flight

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Many animals including birds and humans depend on global visual motion, commonly termed optic flow, to guide movement through natural environments. In all tetrapods, the pretectal nucleus lentiformis mesencephali (LM, which is homologous to the mammalian pretectal nucleus of the optic tract) contains retina-recipient neurons that respond to optic flow stimuli, and demonstrate tuning with respect to direction, speed, and in the spatiotemporal domain. Neural inactivation experiments and electrophysiology paired with eye tracking demonstrate that LM is implicated in image stabilizing optokinetic responses. Given the physiology and connectivity of LM, it is expected to also play a role in the analysis of optic flow during locomotion. However, LM function has never been investigated during natural locomotion. Our foundational hypothesis is that LM neurons are modulated by optic flow during avian flight.

We developed in vivo electrophysiology paired with high-throughput motion tracking to record spiking activity of LM neurons in freely flying zebra finches. Analyses focus on the responses of LM neurons to flight-induced optic flow, head turn-induced optic flow, and exogenous optic flow stimuli presented along the lateral walls of the flight chamber.

We found that most LM neurons preferred forward motion and were suppressed by flight-induced backward motion. However, some neurons could be excited by exogenous forward motion, suggesting LM neurons faithfully report sensory driven optic flow during flight. We also discovered neurons that modulated with optic flow in the frontal region of the visual field and were strongly modulated by frontal expansion optic flow cues during flight.

These findings suggest that LM participates in visuomotor transformations during locomotion, extending its role beyond optokinetic responses.

Poster Session 2 | Poster Wall 223 | Label: PS2.223

Category: Vision and photoreception

Investigating the visual physiology of insect ocelli

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In insects, most visual tasks are completed by the primary compound eyes. However, many groups also utilize a secondary visual system, the ocelli. The ocellar system is composed of two or three single lensed eye spots that reside on the head between the compound eyes, typically forming a triangle. While early studies thought that ocelli were basic light sensors, with no focusing power, spatial detection, or specializations, we now know this is not the case. Ocelli are hypothesized to be used for a variety of visual tasks, the best described being flight stabilization and sky compass orientation. Because of the sophisticated compound eyes of insect systems, the ocelli are seemingly redundant; however, they may be used for quick decisions and rapid processing. While the compound eyes tend to be well matched to the spectral environment an insect inhabits and the ecological needs of the animal, less is known about ocelli. It is likely that, much like the compound eyes, ocelli are specialized for specific functional requirements and therefore have distinct visual sensitivities compared to the compound eye. However, relatively few studies have been completed on the ocelli at this time. To better understand ocellar visual systems we used electroretinogram recordings. We examined multiple aspects of the visual system, including spectral sensitivity, flicker fusion frequency, and irradiance sensitivity of the ocelli in multiple insect species from the orders Hymenoptera, Diptera, and Odonata. For many of these species, we took the same measurements in the compound eye to better understand how the ocelli are sampling visual space compared to the primary visual system. Through this study, we aim to increase our understanding of how the ocellar visual systems are being used as well as how function changes between species, particularly those that inhabit different ecological niches.

Poster Session 2 | Poster Wall 224 | Label: PS2.224

Category: Vision and photoreception

Exploring Visual Motion Neurons in Drosophila

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Flies regulate their flight behaviors using visual motion patterns induced by self-motion. Lobula plate tangential (LPT) neurons, which exhibit response properties well-matched to these optic flow patterns, are presumed to contribute to the optomotor behaviors that stabilize flight. While extensively studied in larger flies, only recent advancements allow for detailed anatomical and functional analyses in Drosophila. However, no LPT neuron has been definitively linked to a specific optomotor reaction. Here, we explore LPT types in lobula plate layers 1 and 2 and their potential roles in optomotor responses.

Using EM reconstructions, we identified 58 LPT types in D. melanogaster and predicted their receptive fields. With these predictions, we designed wide-field visual stimuli and measured the behavioral responses of tethered, flying flies with a high-resolution LED display. We found that the optomotor response of wildtype flies to drifting vertical gratings was strongest for stimuli in front of the fly while lateral stimuli evoked no response. To identify the LPT types underlying this behavior, we targeted LPTs in layers 1 and 2 with innervations corresponding to frontal field of view. We generated candidate split-GAL4 driver lines for these cell types, silenced them by expressing Kir2.1 and compared the behavioral responses to wide-field motion patterns and to those of genetic control flies.

Surprisingly, we have not yet identified any LPT type which, when silenced, impairs specific optomotor turning reactions. Silencing well-known LPTs, the HS and H2, resulted in enhanced turning, despite these cells being excitatory. This suggests potential redundancy with neurons working in concert or reliance on different neurons for these reactions. Ongoing research includes further EM data analysis, alternative silencing methods, and detailed electrophysiological characterization of individual LPT neurons to understand the neural underpinnings of optomotor behaviors.

Poster Session 2 | Poster Wall 225 | Label: PS2.225

Category: Vision and photoreception

Single-cell transcriptomics of a looming-detection neuron in isolated and crowded Schistocerca sheds light on sensory processing for collision-avoidance behavior

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Grasshoppers and locusts have long been used for physiological investigation of neural processing in collision avoidance behavior. Detailed electrophysiological studies on the lobula giant movement detector (LGMD), an identified looming-detection neuron, have determined the functions of voltage-gated ion channels in neuronal computations related to jump escape behavior. These studies have been hindered by a lack of genetic information, limiting channel identification and the use of modern genetic and transcriptomic techniques. The recent release of high-quality genomes for the desert locust (Schistocerca gregaria) and five other Schistocerca species allows the pursuit of genetic investigations. We extracted somas of the LGMD neuron for RNA sequencing from crowded and isolated desert locusts and crowded American bird grasshoppers (Schistocerca americana). The LGMD transcriptome data confirmed the presence of voltage-gated conductances previously characterized using electrophysiology and pharmacology and allowed the identification of the underlying channel types. Solitarious desert locusts showed greatly reduced escape probability compared to gregarious ones or bird grasshoppers. Differential gene expression in the LGMD was explored to find the possible mechanisms of these differences in escape behavior and RNAi probes were generated to target the LGMD-specific transcripts. This research will expand our understanding of the role of voltage-gated ion channels in neuronal computation and the role of differential gene expression in phenotypic plasticity.

Poster Session 2 | Poster Wall 226 | Label: PS2.226

Category: Vision and photoreception

Seasonal plasticity in colour vision of damselfishes and surgeonfishes

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Coral reef ecosystems show fluctuations in their prevailing light environment as conditions can change naturally with diurnal cycles, seasons, depth, or more stochastically due to anthropogenic events such as sediment run-off. Previous research has demonstrated that coral reef fish can adjust their visual system to meet the changing environment they experience. Here, we investigate how the shift in the overall light environment that occurs seasonally amongst coral reefs impacts fish vision. During winter the clear oceanic water has little to no suspended or dissolved matter, causing a broad 'blue' light environment with ample wavelengths ranging from ultraviolet (UV) to red. In contrast, the water becomes murkier and red-shifted in summer as algae and suspended sediments dominate. We used an integrative approach across two dominant coral reef fish families, Pomacentridae (damselfishes) and Acanthuridae (surgeonfishes), to determine the sensory benefit of visual plasticity. Upon molecular analysis, some but not all species can track these seasonal changes to the light environment by altering the expression pattern of their retinal genes including the visual opsin genes. Using electroretinograms and spectral sensitivity modelling, we further investigated if the shifts in opsin gene expression functionally alter reef fish's physiological response and, ultimately, colour vision. Finally, phylogenetic comparative analyses provided insights into significant predictors that underlie seasonal plasticity. Overall, our results show 'natural' plasticity amongst coral reef fishes, whereby ecological differences or phylogenetic inertia may contribute to the species-specific plasticity seen in opsin expression.

Poster Session 2 | Poster Wall 227 | Label: PS2.227

Category: Vision and photoreception

Dissection of the opponent mechanisms for processing skylight cues in the Distal Medulla of Drosophila melanogaster

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The fruit fly Drosophila melanogaster is an important model for understanding the structure and function of the visual system. Its adult eye is composed of ~800 ommatidia, each containing eight neuronal photoreceptors (PRs), expressing a specific Rhodopsin, with two PRs (named R7 & R8) forming long axonal fibers terminating in the medulla neuropil. Drosophila can detect a wide range of visual stimuli, such as light intensity, motion, color, allowing for foraging and mating behaviors. The motion vision system was exhaustively characterized in Drosophila, but the processing of skylight cues and the neural network implicated herein are much less well understood. Drosophila's retinal mosaic includes ~40 ommatidia in the Dorsal Rim Area (DRA), specialized for detecting skylight polarization, whereas the remaining ommatidia (named pale and yellow) have different chromatic sensitivities and are stochastically distributed throughout the retina and serve color vision. Interestingly, similar opponent mechanisms underly the processing of color and skylight polarization. The polarization opponency system for detecting skylight polarization in the DRA operates by subtracting signals from R7-DRA & R8-DRA of the same ommatidium (and vice versa), both being tuned to orthogonal angles of polarization. In contrast, the color opponency of pale and yellow ommatidia is achieved by substraction between R7 & R8 cells expressing different Rhodopsins. At the neuronal, synaptic, and molecular levels, the mechanism of polarization opponency is not fully understood. Our objective is to investigate the DRA neurons Dm-DRA1 and Dm-DRA2, which process polarization opponent signals and potentially integrate them with other celestial cues. This research includes both a connectomic and optophysiological characterization of Dm-DRA1 and Dm-DRA2. Taken together, we aim to elucidate the cellular contributions to opponent coding, as well as to the integration of different skylight cues.

Poster Session 2 | Poster Wall 228 | Label: PS2.228

Category: Vision and photoreception

Probing binocular vision in Drosophila melanogaster

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Many animals use vision to guide spatial behaviors like moving through the environment, interacting with conspecifics, predating, or performing goal-directed limb movements. In primates, a special form of binocular vision, stereoscopic vision (stereopsis), plays a pivotal role in estimating distances and depth perception. Similarly, the praying mantis, a predatory insect, uses stereopsis for distance estimation. In mantises, neurons have been discovered that are presumably involved in neural computations for stereopsis. These cells respond particularly well to the presence of objects seen by both eyes, in particular when the view from the right eye is shifted horizontally by a specific amount compared to the left eye. The sensitivity to such a specific horizontal shift, called binocular disparity, is a hallmark feature of neurons involved in stereoscopic vision.

Several disparity-sensitive neuron types have been discovered in mantises, but understanding how these neurons interact at the network level is challenging. The absence of a complete connectome and the lack of sophisticated genetic tools for these insects contribute to this difficulty.

Therefore, we aim to determine whether disparity-sensitive neurons exist in the genetically tractable fruit fly, Drosophila melanogaster, similar to the cells found in the praying mantis. We developed an experimental paradigm to study the calcium responses of visual interneurons using in vivo two-photon microscopy while presenting 3D visual stimuli to the flies. We will present the first results of our study on binocular computations in the fruit fly brain.

Poster Session 2 | Poster Wall 229 | Label: PS2.229

Category: Vision and photoreception

Body size and light environment modulate flight speed in free flying Drosophila melanogaster

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For flying insects, visual control relies on acquiring an adequate amount of light. But many insects function in circumstances where it is difficult to collect light, including dim environments, viewing high image speeds, or with eyes of relatively modest light gathering power. The size of holometabolous insects is determined by larval diet, and since compound eyes grow in proportion to body size, smaller individuals face visual constrains. To determine the effects of limited light collection on flight structure, we restricted light for vinegar flies in two different ways, placing them in dim light, or with developmentally smaller eyes, then examined their three-dimensional flight paths for several days. We found similar flight deficits manifest in both dim light and small eye conditions, such as decreased flight speed. Through a machine learning approach, we also found non-linear differences in flight dynamics between conditions. To test whether the reduced speed observed in small flies was solely due to their limited light gather ability, we doubled the daytime brightness used in previous experiments and analyzed their flight paths. We found that when more light becomes available, small flies speed up dramatically. This supports the idea that flight changes in smaller individuals result from visual deficits, rather than other elements of body structure.

Poster Session 2 | Poster Wall 230 | Label: PS2.230

Category: Vision and photoreception

The repeated evolution of eye loss in spiders

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Multi-ocular vision has been a feature of the arthropod body plan for more than 500 million years. Spiders have some of the most diverse and successful multi-ocular systems among living arthropods. Although most species have four pairs of eyes, spiders exhibit a huge variety of configurations in eye size, number, and arrangement. Many taxa have lost one or more pairs in independent phylogenetic events, most commonly, but not exclusively, the anterior median eyes (AME). The variation in eye number is of particular interest in the Synspermiata, an early-diverging clade of spiders with multiple reductions and losses of eyes occurring independently in Caponiidae, Dysderoidea, Scytodoidea, and the Lost Trachea Clade. Among these, caponiids are a highly unusual, enigmatic group with many fascinating morphological traits including their highly variable number of eyes: caponiids may have zero, two, four, six, or eight eyes, making this group the most variable of any epigean family. Unusually, it is the AMEs that are most commonly retained in these taxa . However, almost nothing is known about their visual capabilities or ecology. To address these gaps and assess the impacts of eye loss on caponiids, we present the first study of eye morphology in caponiid species and closely related synspermiatan taxa with different eye numbers. We used synchrotron X-ray tomography and a geometric morphometric approach to investigate their structure and quantify the size, position, and orientation on the carapace. We also explored the internal anatomy and compared it between the principal and secondary eyes. Of particular interest are the relocation of eyes on the carapace to retain optimal field of view coverage, compensation through the reallocation of resources to the remaining eyes, and compensation through increased investment in other sensory modes. Preliminary results on the eye number and diameter of the analyzed species are presented and discussed.

Poster Session 2 | Poster Wall 231 | Label: PS2.231

Category: Vision and photoreception

Variable Adaptation Tunes Dragonfly Optic Flow Neurons to Relevant Speed Ranges

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Dragonflies have varied flying behaviour, from stationary hovering to very fast pursuit flight. To gauge their own movement through the world, dragonflies interpret widefield visual motion cues (i.e. 'optic flow'). Neurons responsive to such optic flow, referred to as Lobula Tangential Cells (LTCs), undergo adaptation, modulating their response to continuous motion.

Previous work showed that LTCs share a mutual, naive tuning state, and adapt their tuning properties to different temporal and spatial frequencies. We recorded intracellular electrophysiological responses from LTCs to stimuli with and without preceding adapting stimuli ('test-adapt-test') to determine the causal factors resulting in this 'differential adaptation'.

In most cases, adapting stimuli caused reduction in LTC contrast sensitivity, and the magnitude of reduction was dependent on the strength of response to that adapting stimulus (n=40). In LTCs preferring vertical motion, adaptation reduced contrast sensitivity relatively uniformly across temporal frequencies. In LTCs preferring horizontal motion, responses to lower temporal frequencies adapted strongly, but responses to high temporal frequencies did not adapt, retaining similar neuronal activity despite the strong response to the adapting stimulus. For these horizontal LTCs, this selective resistance to adaptation results in a faster temporal frequency (and thus speed) tuning peak compared with unadapted tuning.

Horizontal LTCs, sensitive to optic flow from dragonflies' extremely fast yaw movements, respond robustly and selectively to such motion when adaptation has shifted their tuning towards faster velocities. Vertical LTCs, sensitive to optic flow from pitch, lift and roll movements of relatively lower speeds, may not require adaptive velocity tuning changes. As such, adaptation-mediated changes to temporal frequency / speed tuning may serve to sensitise LTCs to speeds which are salient to their individual directional preferences.

Poster Session 2 | Poster Wall 232 | Label: PS2.232

Category: Vision and photoreception

Neural representations of distributed vision in the eyed chiton Acanthopleura granulata

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Vision is often conceived as a pair of cephalized eyes and a brain; however, a diverse set of invertebrates view their environment in an unfamiliar way by having many light-sensing organs distributed across their bodies and lacking a stereotypical brain. Visual information integration and processing in animals with distributed visual systems is likely not only novel compared to canonical cephalized visual processing, but may represent a suite of unexplored visual computation strategies. We are exploring distributed visual processing in the chiton (Mollusca; Polyplacophora) Acanthopleura granulata, which has hundreds of small eyes (~80µm in diameter) embedded within its shell plates. From our previous work, we know optic nerves from the shell eyes of A. granulata innervate the central nervous system to form a decentralized visuotopic map along the lateral neuropil. We have followed up on this work by recording light-induced electrophysiological responses in the lateral neuropil, which are the first reported recordings for chitons and confirm that A. granulata integrate and process visual information very differently from other animals by using decentralized circuits around their body. We found that light flashes onto the overlying shell-plates elicit light-off bursting responses in the underlying lateral neuropil. By exploiting these light-induced electrophysiological responses, we found that the visual system of A. granulata has a temporal sampling rate of ~38Hz and has one spectral class of photoreceptors (λmax = 480nm). Additionally, we found that the visual system has a dynamic range spanning 3 log units of light intensity (1.0E15-1.0E18 photons cm^{-2} s^{-1}), and that the response amplitude scales linearly with flash duration until plateauing at ~1.0s flash duration. This work is critical to better understand the fundamental differences between distributed and cephalized vision, which will uncover novel strategies of efficient, decentralized information processing.

Poster Session 2 | Poster Wall 233 | Label: PS2.233

Category: Vision and photoreception

The optomotor response depends on motion direction and varies across the visual field during hovering flight in hummingbirds

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Hummingbirds use visual input to inform positional stability during hovering. When large field stimuli are presented in their frontal visual field, hovering hummingbirds will drift in the corresponding direction. The optokinetic responses (OKR) are the head and eye movements that are used to stabilize the retinal image during motion. The OKR are biased toward temporal-nasal (T-N) motion in most laterally eyed animals studied to date. Hummingbirds also have a nearly 360° visual field, but their fovea is oriented laterally and their area temporalis, which has lower spatial resolution, is oriented forwards. These features led us to ask whether the hummingbird optomotor response varies across different regions of the visual field and whether it has a directional bias during hovering flight.

We addressed these questions by creating a square arena with screens on each wall, and presenting hovering Anna's hummingbirds with various combinations of looming and receding spirals, and a grating of vertical black and white bars drifting in both directions in the horizontal plane. Bird head positions and flight trajectories were recorded using a 3D motion capture system.

We found that vertical black and white bars drifting horizontally in a single plane did not elicit any directional bias, however, two parallel planes elicited a stronger overall response with a slight bias for nasal-temporal (N-T) drift. Looming and receding also provide N-T and T-N motion (respectively). Responses to looming were always stronger than to receding, and responses to both looming and receding spirals were strongest in the frontal visual field. Competing the vertical gratings with looming/receding consistently led to stronger responses to the former. Our results suggest that hummingbirds have relatively uniform responses to different motion directions but may have a subtle bias for N-T rather than T-N motion for both horizontal and looming/receding visual motion during hovering.

Poster Session 2 | Poster Wall 234 | Label: PS2.234

Category: Vision and photoreception

Evolutionary, developmental, and ecological drivers of eye morphology in the modular visual system of spiders

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Visual ecology is the study of an animal's vision in the context of its behaviour and environment. Therefore, when a comparative approach is taken, visual ecology presents some of the best and most salient examples of evolution by natural selection, from which more general and universal principles of evolutionary theory can be tested or unveiled. One particularly promising system for applying this comparative visual ecology approach is the spiders (Araneae). This is because they represent a speciose and ecologically diverse clade, whilst possessing a modular distributed visual system that exhibits a wealth of morphological and functional diversity based on a highly conserved blueprint. The relative size and shape of spider eyes is highly variable at both the interspecies level, indicative of varying physiological investment in vision, and the intraindividual level, indicative of specialisation of different eye pairs. However, whether these variations correlate with the evolutionary history or ecology of each spider species has largely gone untested. Here, we present results to rectify this by utilising x-ray computed tomography and geometric morphometric analysis to quantify the size and shape of eyes of over 40 spider species representing phylogenetically and ecologically diverse clades. From this, we assess the impact that various life history traits, such as predation strategy, association with aquatic environments, sociality, and mimicry, have upon spider eye morphology, in order to better elucidate drivers of sensory system evolution.

Poster Session 2 | Poster Wall 235 | Label: PS2.235

Category: Vision and photoreception

Circuit Implementation of Multimodal Integration in Skylight Navigation

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Many insects utilize various visual cues, including landmarks, chromatic gradients, and natural polarization patterns, for navigation. This capacity is crucial to foraging, mating, and predator evasion. Despite its tiny size, our model system, the fruit fly Drosophila melanogaster, possesses a sophisticated visual system enabling these behaviors. Each optic lobe comprises approximately 800 repetitive units, called ommatidia. Each ommatidium contains eight photoreceptors, named R1 to R8. While R1 to R6 cells express rhodopsin Rh1, R7 and R8 choose between expression of Rh3-Rh6, with sensitivity ranging from UV to green light. Notably, specialized ommatidia in the dorsal rim area (DRA) detect polarized skylight for orientation. Visual information from photoreceptors is relayed to downstream neuropils, including the lamina, medulla, lobula, and lobula plate.

Our research focuses on the Anterior Visual Pathway (AVP), which, across insects, is crucial for conveying polarized light and color information to the central brain for interpretation and the execution of behavioral responses. Through connectomic analysis, we identified Medulla-Medulla bilaterally projection neurons downstream of R7-DRA. Their upstream synaptic targets are not only predicted to be polarization-sensitive but we expect some of them to be color-sensitive. Morphology and connectivity therefore suggest that polarization & color information may be integrated at this early stage along the AVP, further indicating an important role for binoculars.

We use connectomic techniques, as well as immunohistochemistry, to identify split Gal4 lines and lexA driver lines of pre- and post-synaptic targets. Future plans involve using physiology (Calcium imaging) and behavior assays to elucidate how these neurons integrate contralateral and ipsilateral information to inform different fly behaviors. This study aims to deepen the understanding of early-stage binocular integration navigation behavior.

Poster Session 2 | Poster Wall 236 | Label: PS2.236

Category: Vision and photoreception

Recording animal-view videos of the natural world using a novel camera system and software package

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Animal colour vision is very diverse, both in terms of the number of photoreceptor types and their spectral tuning. As a result, each animal perceives colours differently. Although current approaches for studying colours are objective and repeatable, they miss the temporal variation of colour signals entirely. Yet, temporal changes can be central to a visual signal, be those the courtship dances of peacocks or the startle displays of caterpillars. To capture the temporal aspect of visual signals, we developed a novel hardware and software suite, video2vision that allows us to record videos in animal-perceived colours. Specifically, our Python codes transform multispectral photos or videos into perceivable units (quantum catches) for animals of known photoreceptor sensitivity, with R2 > 0.90 accuracy on moving stimuli and under natural lighting. This camera system will enable researchers to explore a wide range of cues and signals in motion, in the natural environments where they are produced and experienced by free-living organisms. Moreover, the plans and codes necessary for end-users to capture animal-view videos are all open source and publicly available to encourage continual community development. Here we debut this system, present tests of its accuracy, and highlight example uses of animal-view videos.

Poster Session 2 | Poster Wall 237 | Label: PS2.237

Category: Vision and photoreception

Polarisation perception in modified ocelli of apoid wasps

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Flying insects are highly reliant on vision for foraging, navigation and the detection of mates. To fulfil their complex visual needs, most have not only two large compound eyes but also three single-lens eyes called ocelli on their forehead. These were thought to work together as a simple light sensitivity sensor, but modern studies have also shown their complex morphology and involvement in flight control and navigation. Apoid wasps, solitary living parasitoids closely related to bees, exhibit dramatic changes to shape and size of the ocelli. Comparable modifications are otherwise unknown in flying insects but have evolved multiple times independently within apoid wasps. While this has attracted great taxonomic interes, the internal morphology and possible functions remained enigmatic.

Modifications range from slight elliptic deformations to C- or comma-shaped lenses. While these are often described as “scars” and “reductions” in the literature, suggesting reduced or missing functionality, my research demonstrates that, despite these strong modifications, the ocelli are still potentially functional, retaining a structured retina and optic nerve. My preliminary data suggests that they might have maintained or even improved the morphological properties to recognize celestial polarisation patterns. In addition to the rhabdom arrangement typical to polarisation perceptive hymenopterans and a comparable rhabdom straightness the ocellar rhabdoms are organised into continuous, distinct lines with seemingly uninterrupted microvilli seams covering multiple cells.

Poster Session 2 | Poster Wall 238 | Label: PS2.238

Category: Vision and photoreception

Behavioral responses of free-flying Drosophila melanogaster to shiny reflecting surfaces

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Active locomotion plays a crucial role in the life of many animals, allowing them to prospect their environment, find essential nutrients, escape predators and find an adequate place for reproduction. Most insect species depend on a combination of visual cues such as celestial bodies, landmarks, or linearly polarized light to navigate or orient themselves in their surroundings. Perceiving this kind of signal, especially the polarized light, and executing a flawless locomotion require a well-integrated navigational and an adapted visual system. In nature, linearly polarized light can arise from the sunlight either being scattered in the atmosphere or from being reflected off shiny, non-metallic surfaces such as leaves or water. Multiple reports have described different behavioral responses of various insects to such shiny surfaces. Our goal was to test whether free-flying Drosophila melanogaster, a well known molecular genetic model organism, also display specific behavioral responses when confronted with such polarized reflections. Fruit flies were therefore placed in a custom-built arena with controlled environmental parameters including temperature, humidity level, white light intensity and reflective background. Flight detections and landings were quantified for three different stimuli: a diffusely reflecting matt plate, a small patch of shiny transparent acetate film, and real water. Considering the fact that the state of hydration may change the motivation of flies to seek or avoid water, hydrated and dehydrated fly populations were compared. Our study demonstrates for the first time that freely flying fruit flies indeed use vision to avoid flying over shiny surfaces.

Poster Session 2 | Poster Wall 239 | Label: PS2.239

Category: Vision and photoreception

Multiple opsin expression in a box jellyfish eye

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Box jellyfish are well-known for their toxic stings, as well as for their unique and surprisingly complex visual system of 24 eyes. While lacking a centralised nervous system, these animals instead possess distinct sensory structures (rhopalia) which each contain six eyes of four different morphological types: two large image-forming lens eyes with spatial resolution of 10 – 20 degrees, and lateral pairs of pigment pit and slit eyes. The slit eyes are particularly intriguing as their visual function is currently unknown. These complex and asymmetrical slit eyes comprise four cell types, with a very weakly refracting lens-like structure. Interestingly, custom antibodies reveal that at least four of the 19 opsins currently known in the box jellyfish species Tripedalia cystophora are expressed in these eyes. We also identify the opsin expressed in the slit eye. We investigate the functional diversity of these opsins, and explore whether this astonishing result can be explained via redundancy, given that cnidarians in general seem to have an extraordinary high number of opsin genes; even the eyeless Hydra vulgaris and Nematostella vectensis have 45 and 29 opsin genes, respectively. By building on our current understanding of Tripedalia opsins, we uncover more of the complexity of the box jellyfish visual system, and of opsin redundancy in Cnidaria as a whole.

Poster Session 2 | Poster Wall 240 | Label: PS2.240

Category: Vision and photoreception

The Evolution of Color Vision in Jumping Spiders

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Shifts from dichromacy to tri- or tetrachromacy offer major leaps in color vision through improvements to color discrimination and expansions in the range of perceivable wavelengths. However, the evolutionary causes and consequences of such transitions remain poorly understood. Here, we describe work to investigate the repeated evolution of transitions in color vision in the principal eyes of jumping spiders (family Salticidae) using a combination of retinal microspectrophotometry, RNA sequencing, and phylogenetic inference across 48 species of jumping spiders spanning the family's phylogenetic diversity. Our results establish UV-green dichromacy as the ancestral state of salticid color vision, and reveal as many as 7 transitions to tri- and/or tetrachromacy. These transitions are not only phylogenetically distinct, they also involve divergent physiological mechanisms. For example, the transition from dichromacy to trichromacy in the Harmochirina and Plexippina involved the evolution of intra-retinal long-pass filters, whereas transitions from dichromacy to tetrachromacy in the Euophryini and Aelurillina were accomplished through the addition of novel photoreceptor types with distinct spectral sensitivities. In some of these cases, we find evidence from RNA sequence data for opsin gene duplication and subsequent neofunctionalization. Such transitions should have important consequences for retinal development, visual function, and neural coding of visual information. In addition, by expanding the range of perceivable wavelengths, such transitions open up opportunities to use a broader palette of colors in foraging and communication. Consistent with this, we provide preliminary evidence that groups with expanded color sensitivities often make more extensive use of color, particularly long-wavelength colors, in courtship signaling.

Poster Session 2 | Poster Wall 241 | Label: PS2.241

Category: Vision and photoreception

Visual projection neurons in the complete connectome of the Drosophila optic lobe

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The nervous system of an adult, male fruit fly has been successfully imaged by Janelia's FlyEM team. Together with a small group of collaborators, we have catalogued all neurons in the optic lobe, including the complete medulla (and accessory medulla), lobula, lobula plate, and approximately half of the lamina. Using first morphology analysis and then iterations of connectivity analysis, we identified and named all cell types of the optic lobe—over 53,000 neurons in ~730 cell types. The categorization of neurons already uncovered many interesting findings and many cell types were described for the first time. In ongoing analysis, we are examining regional variations, and using connectivity to predict both the spatial-visual properties of neurons, such as their putative receptive fields and their "participation" in various pathways. In this direction, we are currently analyzing the full set of Visual Projection Neurons (VPNs) that leave the optic lobe and target various central brain regions. Some of these central brain regions that are involved in vision-based learning are the posterior lateral and ventrolateral protocerebra (PLP and PVLP), mushroom bodies and the central complex. To provide context for functional experiments and understand the organization of visual pathways, we will present an input/output analysis of the most numerous VPN types. We sort the VPNs based on their inputs within the optic lobe to study whether they carry information about motion, ON or OFF signals, spatial-visual features, short versus long wavelengths, etc. Furthermore, by looking into whether the projection neurons target specific central brain areas or combinations of them, we hope to discover how vision is integrated within central brain circuits.

Poster Session 2 | Poster Wall 242 | Label: PS2.242

Category: Vision and photoreception

Luminance-invariant visual processing is a general property of diverse visual system

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For vision to work reliably in different environments, animals must encode visual cues independently of viewing conditions. For instance, visual systems achieve luminance-invariant processing of visual features by implementing luminance gain control, allowing them to compute contrast stably. Early luminance gain control in photoreceptors alone is insufficient, especially in dynamic conditions present in natural environments. In D. melanogaster, stable contrast estimation under such dynamic light conditions is ensured by a luminance gain mechanism implemented in the visual circuitry past photoreceptor, leading to robust behavioral responses. The luminance sensitive second-order neuron L3 is essential to implement luminance gain control on both, fast and slow timescales (Ketkar et al. 2020, 2023). For this purpose, L3 is constantly active in contextual dim light, which is energetically costly. Given that visual environments can differ in their statistics (Frazor & Geisler 2006), we tested if this luminance gain mechanism is present across species or strains, living in different environments.

We show that luminance-invariant behavior generalizes across Drosophila strains and species living in different habitats. Preliminary psychophysics experiments also indicate rapid luminance-invariant processing in humans. Furthermore, D. melanogaster exhibits luminance-invariant behavior under challenging metabolic conditions, e.g. starvation, and maintains this behavior during aging until vision deteriorates, further emphasizing its importance. However, species-specific differences in behavioral responses under bright light conditions can be found, suggesting a species-dependent range of implementing rapid luminance gain control. We are currently establishing transgenic models for cell-type specific physiological recordings in different Drosophila species. This will reveal how the neuronal mechanisms underlying luminance-invariant contrast estimation are shaped across species.

Poster Session 2 | Poster Wall 243 | Label: PS2.243

Category: Vision and photoreception

Improved semi-automated eye tracking in freely moving animals

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Reconstruction of eye views from freely moving animals performing ethologically relevant behaviors requires high-resolution eye tracking. Miniaturization of cameras to be small enough to mount on the head has allowed measurement of eye rotations in all three rotational axes from both eyes while freely moving animals perform visually based tasks. From these recordings, eye models using the characteristic change in pupil shape as the eye rotates, combined with accurate quantification of head position, have enabled quantification of gaze directions relative to the animal. In turn, these gaze directions can be combined with digital reconstruction of the animals' surroundings to generate animals eye views. To improve on the existing tracking methods, we developed an eye tracking technique that incorporates compensation for camera-lens distortions and that reduces the requirement for human intervention in pupil tracking. The approach leverages off a deep learning segmentation tool, YOLO, along with traditional segmentation techniques which, in contrast to marker-based tools, extract even partially visible pupils. For gaze estimation, we refined an existing method by calibrating the eye camera and employing a full perspective camera model instead of an approximate weak perspective model, to calculate eye model parameters and estimate eye rotations from the extracted pupils. Validation, through simulation, showed improved accuracy compared to our previous weak perspective model, with the current method having a factor of 2-3 less error. With this approach, we successfully tracked pupils and estimated gaze direction in freely moving rats, mice, and freely flying hawks.

Poster Session 2 | Poster Wall 244 | Label: PS2.244

Category: Vision and photoreception

Evolutionary remodeling of the primate visual cortex

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Nervous systems incur great costs in an animal’s metabolic energy budget and their sensory periphery often exhibits functionally optimized energy allocation. In the central nervous system, however, evidence for such optimization has remained elusive. Here, we use the partitioning of primate visual cortex (V1) into cytochrome oxidase blobs and interblobs with blobs exhibiting higher activity and metabolic demand. We determined the optimal positioning of blobs relative to V1’s system of orientation domains and tested optimal positioning in a large and phylogenetically informative sample of primates. Blobs were positioned near optimally in trichromatic Macaque but not in color-blind primates. Our results provide unambiguous evidence of metabolic optimization in primate neocortex and that its metabolic compartmentalization can be functionally optimized over evolutionary time scales.

Poster Session 2 | Poster Wall 245 | Label: PS2.245

Category: Vision and photoreception

Sensing the change: bumblebees decide when to forage during the low light conditions of sunrise and sunset

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Only a few diurnal animals, such as bumblebees, extend their activity into the time around sunrise and sunset when illumination levels are low. Low light impairs viewing conditions and increases sensory costs, but whether diurnal insects use the predictable gradual change in low light at the end and start of the day as a cue is uncertain. We observed bumblebee departures from the nest during sunrise and sunset when daylight naturally changes, either increasing or decreasing in intensity. In brighter light bees departed with minimal delay, as expected. In low light the probability of non-departures increased, as bees attempted to return after spending time outside the nest, whilst others delayed their departure, especially in the evening before darkness. This suggests that bees have an opportunity to assess light conditions once they reach the nest exit and decide whether to depart. When bees were allowed to forage in the field, departures dropped in frequency during sunset. Foragers returning in low light carried only partial pollen loads. Also, their pollen sacs contained pollen from the same flower taxa that were exploited in the daytime. Most likely, they visited known locations during the evening opportunistically exploiting profitable flowers. Our findings indicate that changing and low light levels at either end of the day can be keys factors that influence a bumblebee's individual decision of when to start or cessate foraging.

Poster Session 2 | Poster Wall 246 | Label: PS2.246

Category: Vision and photoreception

Group size discrimination in zebrafish: A study of the role of visual dynamic features

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Zebrafish are social animals that live in groups to protect themselves from predators and to access information about food. Costs and benefits vary with group size and zebrafish can discriminate shoals of different sizes. Moreover, they often prefer to join larger groups. Several studies have investigated the influence of types of motion (e.g., biological motion) on the social drive as well as the ability to discriminate the number of static stimuli or live conspecifics. However, the role of motion in assessing group size has not been dissociated from other variables, such as the density or the number of elements. This study investigated the influence of various visual dynamic features on group size preferences. We created artificial visual stimuli (dots) that were moving and varied in several visual variables such as the number of elements, the total amount of displacement, speed or type of movement. Stimuli presentation and data acquisition were automatized and fish preference was tested in a two-choice test. Fish preferred larger groups of dots when the ratio was 1:3. They showed a preference for dots that moved faster, with this preference increasing with the number of dots. Interestingly, a strong effect of the amount of displacement was evident when stimuli differed in small speeds. Furthermore, fish preference for larger groups was strongly modulated by differences in speed between the two groups. This work contributes to elucidating the mechanisms used by zebrafish to assess the quantity of live biological entities.

Poster Session 2 | Poster Wall 247 | Label: PS2.247

Category: Vision and photoreception

Simulating a dynamic vision sensor to compare moving and static photoreceptors

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The stereo range of fruit flies is limited due to the small distance between their compound eyes, which raises the question of how they can extract depth for greater distances. Recent findings in Drosophila research have revealed that individual photoreceptors in the fly eye move in response to changes in light intensity. These microsaccades enhance resolution and potentially augment depth perception. To explore how this local motion contributes to depth perception in the fly brain, we simulated a dynamic vision sensor (DVS) camera, specifically the Davis346, and used the generated event data as input for a simplified photoreceptor model.

DVS cameras, also known as event-based cameras, feature pixels that operate independently, with each pixel reporting changes in brightness as they occur with microsecond temporal resolution. The output of DVS cameras is thus an asynchronous stream of events, comprising the timestamp and pixel address of the event. These characteristics enable DVS cameras to track objects more accurately than conventional cameras and perform better under poor lighting conditions, rendering them particularly suited for simulating the compound eye.

In our simulations, we varied the distance (1-10cm) of the DVS camera to the simulated objects as well as the size of the objects (1.7-3.7va), generating event stores for static and moving photoreceptors. Each photoreceptor's output is determined by the sum of events within its receptive field, gradually decaying to its neutral potential over time. In the dynamic condition, events trigger photoreceptor movements, shifting the receptive field to the left or right. Our comparisons of photoreceptor outputs for different object distances and sizes revealed qualitative differences between static and moving photoreceptors. Currently, we are analysing these differences to identify signatures of specialized neurons that respond to specific distances and potentially improve depth perception range.

Poster Session 2 | Poster Wall 248 | Label: PS2.248

Category: Vision and photoreception

Stress increases the resolution of bee vision

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Emotions orchestrate a range of adaptive behaviors essential for the survival of many species. In humans, for instance, fear notably enhances visual acuity, priming individuals for quicker responses to imminent threats. This study explores whether similar mechanisms are present in species with less complex neural structures—specifically bumblebees, which are known for exhibiting emotion-like states and relying heavily on visual cues for decision-making. Using a Y-maze, bees were trained to differentiate between sinusoidal gratings of varying orientations. Subsequently, half of the bees were subjected to a stress-inducing manipulation—shaking—to elicit an emotion-like state, while the other half served as an unmanipulated control group. The results demonstrate that negative states alter how bees 'see'; specifically, stressed bees showed decreased contrast sensitivity but enhanced resolution thresholds for high spatial frequencies. This suggests that stress-induced states may refine the bees' ability to discern fine details, thereby enhancing their detection of subtle, potentially life-threatening environmental cues, such as camouflaged predators. Contrary to the typically expected speed-accuracy trade-off, bees that experienced stressful event also made faster and more accurate final choices when presented with patterns at high spatial frequencies—a potential indicator of reduced visual noise due to emotional states. This study highlights the potential universality of emotion-elicited sensory adaptations, underscoring the evolutionary benefits of emotional states in driving behaviour.

Poster Session 2 | Poster Wall 249 | Label: PS2.249

Category: Vision and photoreception

Colour blinding Nemo: the development and application of CRISPR/Cas9 and single-cell RNA sequencing to study vision in the false clownfish, Amphiprion ocellaris

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Understanding the genomic underpinnings of animal traits lies at the centre of much biological exploration. However, a lack of reverse genetic technologies has made studying gene function in marine animals difficult, if not near impossible. This changed with the recent development of the CRISPR/Cas9 and related gene-editing technologies, promising, for the first time, an easy-to-use and affordable way to manipulate the genomes of non-model animals. At the same time, modern sequencing technology has reached a level at which we can now study the expression of genes in single cells, maintaining spatial information and linking gene function to cell type, connectivity, and mechanism. Here, I will present our recent efforts in developing a CRISPR/Cas9-based protocol to engineer coral reef fishes genetically. Using the false 'Nemo' clownfish, Amphiprion ocellaris, as our study animal, we show the successful application of CRISPR/Cas9 to knockout or genetically ablate two separate target sites in the first generation: the rhodopsin-like 2B opsin gene (rh2b) involved in vision, and the Tyrosinase-producing gene (tyr) involved in the production of melanin. Using brightness discrimination tasks, we show that rh2b deficient Nemos are worse at detecting green light, while violet/blue and red detection remains unaffected. snRNA-seq further revealed a re-shuffling of the retinal cell populations. Hence, the high efficiency with which CRISPR/Cas9 operates can be leveraged to study gene function in animals with long generation times. We aim to inspire more scientists to adopt these latest molecular technologies for their study systems by discussing the many challenges we faced (and are still facing) and the solutions we came up with.

Poster Session 2 | Poster Wall 250 | Label: PS2.250

Category: Vision and photoreception

Exploring visual feature space in Drosophila melanogaster

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Visual processing centres in animal brains are tasked with the job of transforming patterns of light falling on the retina into interpretable, actionable visual features. In Drosophila melanogaster, several ethologically relevant visual features—such as those produced by a rapidly approaching predator or by a prospective mate—are encoded by LC (lobula columnar) neurons. The ~20 anatomically distinct LC types each comprise a population of neurons with dendrites that tile retinotopic space in the optic lobe and axons that converge on a cell-type-specific glomerulus in the central brain. Combined with functional data, this striking anatomical arrangement suggests that the optic glomeruli are a crucial information bottleneck where many key visual features are encoded. However, the tuning of many LC types has been only partly described. We will present preliminary work on a computational pipeline for calcium-imaging data that aims at discovering maximally activating visual stimuli for neurons. We plan to use this approach to perform a systematic investigation of the tuning of LCs and selected downstream partners. Our work will shed light on how visual features are encoded and processed in the brain of Drosophila melanogaster.

Poster Session 2 | Poster Wall 251 | Label: PS2.251

Category: Vision and photoreception

Polarization vision as a source of visual contrast in crabs

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The usage of polarized light to enhance object or motion detection, termed object-based polarization vision, has been recognized in a number of animals inhabiting intertidal and aquatic environments. However, the visual computations and neural mechanisms underlying such capabilities remain unknown.

In recent years we have studied the sensitivity to polarization contrast in the grapsid crab Neohelice granulata, a semiterrestial crab that inhabits intertidal estuaries and salt marshes. We quantified the escape response and the changes in heart rate of animals evoked by looming disk stimuli with an 80° polarization difference between the object and the background. Most animals responded by freezing or escaping. By co-rotating the e-vector of light from object and background we found that the escape response varied periodically with a 90º period. Maximum responses were obtained for object and background e-vectors near the vertical and horizontal orientations. Cardiac responses matched these results. Salt marshes produces horizontally polarized light reflections, thus, our results provide experimental evidence that Nehoelice perform a two-channel (vertical/horizontal) computation to achieve object-based polarization vision maximizing sensitivity in its natural environment.

To study how polarization contrast is combined with intensity contrast information we modified a 3D monitor to modulate differentially the intensity of vertically and horizontally polarized light. We confronted animals with edge motion stimuli of different intensity contrasts, with or without polarization cues, and quantified their escape response and heart rate activity. For both type of responses the addition of polarization cues to an intensity contrast stimulus enhanced responses only when intensity contrast was low (Michelson contrasts bellow 0.30). These results suggest that, in nature, polarization information can effectively enhance visual contrast of moving objects under dim light conditions.

Poster Session 2 | Poster Wall 252 | Label: PS2.252

Category: Vision and photoreception

Molecular and functional organization of the octopus visual system

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Cephalopods have a sophisticated visual system that underlies a wide array of visually guided behaviors. However, because their brains evolved independently from those of other highly visual species, the neural organization of their visual system is dramatically different, and relatively little is known about its molecular and functional organization. We are using multiple approaches to explore the visual system of Octopus bimaculoides. We conducted single cell RNA sequencing and in situ hybridization of the optic lobe to identify and localize molecularly distinct cell types within it. These revealed classes of cells delineated by differential expression of neurotransmitters, neuropeptides, and developmental genes, with a detailed laminar organization. We also developed a two-photon calcium imaging protocol to identify basic neuronal response properties in the cephalopod central brain. With this, we identified areas in the optic lobe with spatially localized receptive fields that are organized retinotopically. We also found the emergence of size selectivity across optic lobe layers, with distinct response properties to ON or OFF stimuli. We are now using this imaging approach to explore responses to more complex visual features, such as motion and orientation. Linking together the molecular and functional organization of the octopus optic lobe should provide new insight into how the neural circuits of their visual system enable their wide range of remarkable behaviors.

Poster Session 2 | Poster Wall 253 | Label: PS2.253

Category: Vision and photoreception

Neuroanatomy of the visual pathway of the harbour seal (Phoca vitulina) brain

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Pinnipeds, as semi-aquatic mammals, exhibit profound anatomical adaptations to facilitate efficient underwater locomotion. While their overall morphology and physiology is fairly well-described, hardly anything is known about their brain. This study aims to elucidate adaptations of sensory systems, notably the visual pathway, in harbor seals (Phoca vitulina), to their aquatic habitat. Employing methodologies encompassing blockface imaging to map gray and white matter structures whole-brain-wide, 3D Polarized Light Imaging (3D-PLI) to characterize the fiber architecture, and cell-body staining to describe the cyto-architecture, we seek to discern specialized neuroanatomical features that might reflect semi-aquatic adaptation. Our analyses revealed a different microstructure of the lateral geniculate nucleus (LGN) and the visual cortex, which are both core regions of the visual pathway. For example, the Gennari stripe of the visual cortex which is known to receive afferents from the LGN, was less prominent as compared to humans and vervet monkeys. The LGN seems to reveal a different laminar pattern as compared to other mammals. Moreover, we did some proof-of-concept labeling in the blockface images to reveal the gross-anatomy of selected nuclei and parts of the visual pathway. Our final goal is to detail the characteristics of the entire visual system of the harbour seal, exploiting the complete wealth of the collected high-resolution multimodal histological data. Collectively, our findings advance understanding of the neuroanatomical adaptations in semi-aquatic species and offer insights relevant to behavioral and neurophysiological studies.

Poster Session 2 | Poster Wall 254 | Label: PS2.254

Category: Vision and photoreception

The synaptic complexity of a lobula giant neuron in crabs

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Arthropods are diverse, abundant, successful animals that exploit all available ecological niches. They sense the environment, move, interact with prey/predators/conspecifics, learn, etc. using small brains with 5 orders of magnitude fewer neurons than mammals. Hence, these microbrains need to be highly efficient in information processing. One distinct aspect is the presence of large, easily identifiable single neurons that act as functional units for information processing integrating a high volume of information from different sources to guide behavior. To understand the synaptic organization behind these high integration nodes research on suitable neurons is needed. The lobula giant neurons (LG) found in the third optic neuropil, the lobula, of Neohelice granulata, respond to moving stimuli, integrate information from both eyes, show short- and long- term plasticity and are thought to be key elements in the visuomotor transformation guiding escape responses to approaching objects. One subgroup, the MLG1 (Monostratified Lobula Giants type 1) possess wide main branches and a regular arrangement in a layer of the lobula that allows their identification even in unstained preparations. Here, we describe the types and abundance of synaptic contacts involving MLG1 profiles using transmission electron microscopy (TEM). We found an unexpected diversity of synaptic motifs and an apparent compartmentalization of the dendritic arbor into two domains where MLG1s act predominantly as presynaptic or postsynaptic, respectively. We propose that the variety of contact types found in the dendritic arbor of the MLG1s reflects the multiple circuits in which these cells are involved. Regarding collision detecting pathways, they are probably similarly organized in crabs and locusts.

Poster Session 2 | Poster Wall 255 | Label: PS2.255

Category: Vision and photoreception

RECORDING FROM IDENTIFIED VISUAL NEURONS IN A MOVING CRAB

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Recent discoveries in vertebrates and invertebrates highlighted the modulatory effects of locomotor activity on the activity of visual neurons. The crab Neohelice is a recognized model for investigating the neural basis of visually guided behaviors. Five different classes of lobula giant (LG) neurons are thought to conform to a chief microcircuit involved in the visual control of the crab´s escape. The connections between each of these neurons with different aspects of behavior have been established by matching the temporal course of behavioral responses to a variety of visual stimuli, with intracellularly recorded responses to the same stimuli obtained from immobile individuals, i.e., behavioral and neuronal responses were obtained separately from different individuals. Recently we succeeded in making tetrode recordings in animals performing their locomotor activity on a treadmill device. The differential preferences of the distinct LG classes for particular visual features established in our intracellular studies allowed us to disclose the identity of units recorded extracellularly. Thus, we can now simultaneously record behavioral and electrophysiological responses from identified neurons to a variety of visual stimuli, such as looming stimuli, flow field patterns and small moving objects. Using the simultaneous recording approach, we confirmed several of our previous conclusions derived from separate behavioral and intracellular experiments, such as the correspondence between LG plastic changes and behavioral changes that occurred during repeated stimulus presentations. In agreement with recent findings from few other animals, we found that the activity of LG neurons of the crab is modulated by the locomotor activity of the animal. Our results illustrate the importance of recording from well identified neurons in a moving animal.

Poster Session 2 | Poster Wall 256 | Label: PS2.256

Category: Vision and photoreception

From night stalkers to day walkers: Diurnal foraging in a nocturnal predator, the Ogre-faced spider

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Animals benefit from possessing sensory systems well-suited to their environmental niche. However, the enhancement of a sensory system to perform well in one specific niche may come at the cost of performance in other niches. For example, nocturnal animals with visual systems well-adapted for dim light environments may struggle to perform well in the bright light of day. A textbook example of this is the ogre-faced spider (Asianopis subrufa). Pioneering work by Blest, Land, and colleagues first illustrated the supreme sensitivity of ogre-faced spider eyes, while also documenting a daily cycle in retinal morphology and physiology, where photoreceptors degrade at sunrise and resynthesize at sunset. These circadian sensory alterations match activity patterns of predatory behavior, where A. subrufa transform into motionless stick-mimics during daylight hours, only becoming active hunters following sunset. Unsurprisingly, the ogre-faced spider has been a posterchild for visually mediated nocturnal behavior. Here, contrary to foundational work, we show present-day A. subrufua are facultatively diurnal in foraging behavior. First, we report the frequency and ecological correlates of diurnal foraging in natural populations. We then show the effects of diet and lighting environment on the propensity for A. subrufa to forage during the day. Lastly, we compare physiological and behavioral thresholds of predatory behavior between diurnally and nocturnally active spiders. Together, our work illustrates the potential causes and consequences of this enigmatic shift from strictly nocturnal to facultatively diurnal behavior in a visual specialist.

| Poster Wall | Label:

Category: Methods and education

Light microscopy-based dense connectomics in invertebrates

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Animal behaviors are mediated by expansive networks of cellular and molecular components. Mapping the structure of these networks is essential to a complete understanding of the neural basis of behavior. The synaptic structure of densely labeled cellular networks can be reconstructed from nanoscale resolution image volumes acquired using electron microscopy (EM). However, EM does not capture molecular labels and requires specialized equipment not available in many research settings. Light microscopy enables molecular labels to be identified in large image volumes using commonly available instruments, but resolution and tissue contrast limitations have prohibited cell-type-specific molecular localization and synaptic connectivity mapping in densely labeled cellular networks. Recent work in mammals has demonstrated that new techniques in expansion microscopy can be utilized to reconstruct densely labeled circuits with synaptic resolution and molecular contrast by acquiring nanoscale brain image volumes with EM-like ultrastructural contrast using conventional light microscopes. Here, we adapt and optimize this approach for invertebrates, with an initial focus on Drosophila. We utilized this approach to rapidly acquire whole brain ultrastructure image volumes with effective lateral resolutions of 10 nm. We demonstrate our ability to simultaneously label brain ultrastructure and molecules of interest, segment individual neurons, and to identify cellular organelles and synaptic connections. This work enables dense circuit connectomics with molecular contrast to be utilized to study the neural basis of natural behaviors in a wide range of animals without the need for genetic tools or specialized equipment.

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