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ABSTRACT BOOK

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↑UP

ORAL COMMUNICATIONS

MONDAY, 25 JULY 2022

PRESIDENTIAL SYMPOSIUM

PS1

Specializations in optic flow encoding in the pretectum and accessory optic system of hummingbirds and zebra finches

Doug Altshuler[1]; Andrea Gaede[2]; Graham Smyth[1]; Vikram Baliga[1]; Cristian Gutierrez[3]; Doug Wylie[3]
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Optic flow is processed in evolutionarily conserved midbrain pathways containing retinal recipient nuclei. In birds, these nuclei are called the lentiformis mesencephali (LM) and the nucleus of the Basal Optic Root (nBOR). A key question is, to what extent are optic flow neurons specialized to species-specific behaviors? Hummingbirds use optic flow to guide hovering flight and previous work has demonstrated that their LM has three unusual features: 1) it is greatly hypertrophied, 2) its neurons are tuned to fast velocities; 3) it lacks an overall direction bias, in contrast to other tetrapods, in which the majority of LM neurons prefer forwards motion. Here, we first asked if the hummingbird nBOR has shifted concomitantly in direction and velocity preference. Extracellular recordings from nBOR in hummingbirds and zebra finches revealed no differences in either direction or velocity preferences. However, as with the LM, nBOR neurons in hummingbirds are more tightly tuned to stimulus velocity. We next asked if the difference in velocity tuning was due to preference for lower spatial frequencies, higher temporal frequencies, or both. Optic flow neurons in both the LM and nBOR of hummingbirds were tuned to much lower spatial frequencies and were more tightly tuned in the spatiotemporal domain. These multiple changes to optic flow encoding in hummingbirds suggest a high degree of specialization for this visual signal. The electrophysiological response properties are consistent with the behavior of hover- feeding in dense foliage, in which self-motion would result in fast movements of large images on the retina.

PS2

Vision and signaling behavior in cleaner shrimp-client fish mutualisms

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Cleaner shrimp are small tropical crustaceans that clean their reef fish clients by removing ectoparasites. Although many client species eat crustaceans, cleaner shrimp are rarely eaten during cleaning interactions. In this talk, I discuss evidence supporting the hypothesis that cooperation between cleaners and clients is mediated by visual signals. I first discuss the visual capabilities of each party, and then apply those measures of visual capability to *in situ* interactions between cleaner shrimp and client fish in two cleaner species—the Caribbean *Ancylomenes pedersoni* and the Indo-Pacific *Lysmata amboinensis*—to examine how they may appear to their mutualistic partners. Network analysis approaches show that certain cleaner and client behaviors function as visual signals. In *A. pedersoni*, we found that signaling by both parties, but primarily the cleaner, is necessary to initiate cleaning. In *L. amboinensis*, we found that cleaners adjust their signaling and cleaning behaviors when interacting with predatory versus non-predatory clients, in a way that minimizes potential risks. Despite being in different families and living in distinct parts of the globe, both species possess conspicuous white antennae that play a role in signaling. In both species, the antennae broadly reflect 40-65% of light, and scanning electron microscopy revealed the antennae have a layer of densely packed nanoparticles 300-400nm in diameter, which likely have a high refractive index. Optical modeling showed that the nanoparticle layers are well sized to increase reflectance, enhancing the brightness of these important signaling structures. I briefly touch on future work on

this system, which is poised to become a model for studies of the evolution and dynamics of interspecific signaling.

PS3

The mind of a bee

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Most of us are aware of the hive mind—the power of bees as an amazing collective. But do we know how uniquely intelligent bees are as individuals? In this lecture I will explore the bees' remarkable cognitive abilities. You will learn that they are profoundly smart, have distinct personalities, can recognize flowers and human faces, exhibit basic emotions, count, use simple tools, solve problems, and learn by observing others. They may even possess consciousness. I will take you deep into the sensory world of bees, and illustrate how bee brains are unparalleled in the animal kingdom in terms of how much sophisticated material is packed into their tiny nervous systems. I also examine the psychological differences between individual bees and the ethical dilemmas that arise in conservation and laboratory settings because bees feel and think. Exploring an insect whose sensory experiences rival those of humans, I will explore the singular abilities of some of the world's most incredible creatures.

PS4

How behaviors evolve

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Striking behavioral diversity has evolved even among closely related animal species, but still little is known about either the genetic or neural mechanisms underlying species-specific behavior. In this talk, I will first introduce our "model clade", wild deer mice (genus *Peromyscus*), and the striking diversity in innate behaviors that have evolved both within and between these species in response to differences in their local environments. I will discuss what we are learning about how both the ultimate (why) and proximate (how) mechanisms driving behavioral variation and ultimately what discovering the connections between genes, neural circuits and behavior can teach us about the evolution of natural behavior.

PS5

Chasing the molecular bases of migratory orientation and magnetoreception in monarch butterflies

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Animal migration has evolved as a critical behavioral adaptation for survival in a wide range of taxa and is characterized by a seasonal movement to escape unfavorable conditions. Migratory species use a variety of sensory cues that allow them to travel long distances in a particular direction. The molecular and neurobiological bases of migratory behavior remain however poorly understood. Catalyzed by the sequencing of a draft genome and the development of reverse genetics tools, the monarch has emerged as a promising model system to drive the fields of migration and magnetoreception forward. Eastern North American monarch butterflies undergo a seasonal migration, where the same individuals accomplish the trip to, and back from, their Mexican overwintering sites. The main strategy used to fly south in the fall and north in the spring relies primarily on a bidirectional time-compensated sun compass. Importantly, the cue that triggers the switch in orientation from south to north in individual butterflies has been identified as sustained exposure to overwintering-like coldness, suggesting a role for environmentally induced epigenetic regulation of flight orientation. Migratory monarchs can also sense and orient to the inclination of the Earth's magnetic field. Although the use of a magnetic compass for navigation, either as a backup system to sun compass or as a fine-tuning mechanism, remains to be established, magnetosensing in the monarch can be leveraged to decipher the molecular underpinning of this enigmatic sense. In this talk, I will present our current efforts to identify i) the molecular correlates of cold-induced flight orientation reversal, and ii) the molecular bases of magnetosensing via the use of a new behavioral assay combined to reverse genetics.

PS6

High-speed decision making in hunting archerfish

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Archerfish have one of nature's most remarkable hunting techniques: they down aerial prey with a precisely aimed jet of water fired over considerable distance. Among the many abilities that come packaged with this behaviour is a very rapid and yet intriguingly complex decision that the fish need to make quickly and accurately to secure prey even when they are heavily outnumbered by competing other surface feeding fish in the wild. Based exclusively on information that is taken briefly after the onset of prey motion the fish select a rapid C-start that turns them right to the later point of catch and sets the appropriate speed to arrive at just the right time. The start-decisions are not hardwired, show no speed-accuracy tradeoff and – for ballistically falling prey – allow the fish to respond accurately to any combination of the initial variables of prey movement as well as its own position and orientation. I will introduce key aspects of this decision and will highlight recent findings on how the decision is tuned to how objects fall.

PLENARY SESSION 1

Stereoscopic vision in the praying mantis

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Stereoscopic or 3D vision is the ability to derive depth information by comparing information from the two eyes. This was long assumed to be such a complex visual ability that it must be limited to large-brained animals with front-facing eyes, such as primates. Samuel Rossel’s 1983 demonstration of stereopsis in an insect, the praying mantis, overturned such assumptions and raised fascinating questions about how insect brains achieve stereopsis and how their stereopsis differs from our own. In this talk, I will review what is known about praying mantis stereopsis, the underlying neurophysiology and the computational algorithms which achieve it.

TUESDAY, 26 JULY 2022

PLENARY SESSION 2

Brain-body interactions underlie flexible and rapid control of walking in Drosophila

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Without a continuous monitoring of movement, intended movements are executed as expected only rarely. This is because of the unpredictable nature of natural environments, and the presence of noise within sensorimotor circuits. To correct movements, sensorimotor circuits must estimate self-motion with high precision to adjust maneuvers at a timescale specific to the task, and at a proper context that is specified by the ongoing task or behavioral goals of the animal. Here, I will discuss our attempts to understand the functional organization of circuits controlling such movement adjustments in the context of a fly exploring a novel environment. We found that recurrent interactions between premotor circuits in the brain and the ventral nerve cord of the fly —the insect analogue of the spinal cord, support rapid and context-dependent steering adjustments that are critical to maintain heading and a stable gaze when the fly intends to do so. Ascending signals from the ventral nerve or vertebrate spinal cords have been observed in premotor circuits in different animal species, but their nature and functional role has remained unclear. Our findings in flies highlight a critical role for these multimodal ascending signals on motor context-dependent computations supporting the coordination of goal-directed movement across the body.

INVITED SYMPOSIUM 1 – THE NEURAL BASIS OF COLLECTIVE BEHAVIOR

The emergence of novel group-level behaviors has been described in terms such as "swarm intelligence" or the "mind of the swarm", referring to the congruence in behavior of swarms composed of many different individuals. Quintessential examples include, swarms of locust, schools of fish, flocks of birds, human crowds, and even artificial autonomous agents (swarming robots). The challenge lies in deciphering and connecting the dynamic interactions between the behavior of individuals, the coordinated activity of group or crowds, and the environment. Specifically, very little knowledge has been gained regarding the neural basis of the decision making and behaviour of the individual, allowing coordination and synchronization among the group. This symposium will comprise very recent findings, presenting novel insights into this important aspect of collective behavior. Advances in the study of collective behavior have always been the result of interdisciplinary collaborative efforts, where experimental work combines with theoretical modelling, and both support engineering endeavors. Accordingly, the research presented in this symposium will include various techniques, from electrophysiology, via virtual reality and robotics, to molecular biology.

S1.1

Regulating cooperative behavior

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Cooperative behavior is a mysterious and experimentally difficult area of biology. The study of cooperative behavior is extremely difficult as it necessarily scales from the function of molecules at individual neuronal synapses, to circuit function, to individual animal behavior and onto eventual group dynamics. During a critical developmental period of the *Drosophila* larval visual system, presynaptic boutons from the eight Rh6 photoreceptor neurons become plastic and increase connectivity to two downstream target neurons. This results in visually driven cooperative feeding behavior and increased fitness in the resultant adult flies. This is being investigated on two fronts: how cooperation occurs amongst blends of larvae with different behaviors and the cellular nature of the late critical period driven synaptic plasticity. To look at how cooperation occurs with blends of animals, a collection of backcrossed wild-caught *Drosophila melanogaster* and allied species were obtained. All cluster and co-cooperate together with a wide variety of successful and unsuccessful interactions. For plasticity, the focus is on an unusual and specialized glial cell which is necessary for synaptic growth.

S1.2

Perception and integration of multiple simultaneous visual inputs in locust swarming

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Swarming locusts are a quintessential example and a successful research model of animal collective motion. The swarm's synchronized mass-movement is dependent on local interactions among individuals. Visual inputs are suggested to play a crucial role in the swift decision-making required for the formation and maintenance of the swarm within the complex and noisy visual surroundings. We presented carefully controlled motion-visual stimuli to individual locust nymphs in a normal walking posture, tethered to an airflow trackball, and monitored their responses using video tracking. Electrophysiological recordings from the descending contralateral movement detector (DCMD) interneuron, a well described motion sensitive visual pathway, were used to complement the behavioral tracking and explore the processing of visual inputs. The observed behavioral responses revealed the use of a match filter in the visual system: a speed threshold, filtering out stimuli with speed beneath that of a walking conspecific. A clear threshold was also evident in identifying motion coherence levels or common group direction, which is suggested to further contribute to swarm integrity. The simultaneous presentation of stimuli of various sizes revealed a tendency for preferred alignment with larger stimuli, probably reflecting increased attention to neighboring locusts over distant ones. Neurophysiological investigation further supported our findings, revealing a clear dependence of the DCMD responses on the visual stimuli motion speed, size, and coherence level. Our results emphasize the instrumental role of visual perception in the locust's collective-motion-related decision making, and offer insights into the complex cognitive mechanisms underlying visual processing within a cluttered environment.

S1.3

Cortical coding of communication calls serving social interactions

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For many animal species, including humans, vocal communication is central for transferring information between individuals and coordinating behavior. In zebra finches for instance, it takes two parents to rear young, and vocal communication is key to coordinate clutch brooding and chicks feeding. In its simplest form, vocal communication is the encoding of a message in an acoustic signal by the emitter and the decoding of that message by the receiver. The roles of sender and receiver can alternate between individuals, resulting in an emergent feedback loop that governs the behavior of both. I will describe several lines of research that are beginning to reveal the neural mechanisms that underlie the reciprocal exchange of information in communication. First, we can crack the acoustic code of vocalizations to identify the nature of the message as the identity, intentions, emotional states and/or position of the vocalizer. Then, studying the neural activity in vocalizing animals reveals the motor pathways that generate the motor command for the production of the signal and in particular uncovers the implication of the telencephalon in this motor task. I will show some very recent data that illustrate how the motor cortex is engaged in the production of vocalizations in the Egyptian fruit bat. On the perceptual end, tracking neural activity in the auditory regions of receivers demonstrates what neural computations are performed to decode the different components of the message. I will use findings in zebra finches to illustrate how forebrain neurons decode an entire repertoire.

S1.4

Zebrafish shoaling: Visual recognition of conspecifics by a tecto-thalamic neural circuit

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Many animals live in groups, held together by a basic social affiliative drive that requires detection and approach of conspecifics. Social affiliation is a prerequisite of interactions such as aggression, mating or play, and a proximal cause of swarm, flock, and herd formation. Shoaling in teleosts is governed by at least two local rules, long-distance attraction and short-distance repulsion. While neural mechanisms underlying social behaviors have recently gained much attention, we know relatively little about the sensory detection of social signals (beyond pheromones). Self-like biological motion is a potent visual trigger for social affiliation in juvenile zebrafish (Larsch and Baier, 2018). By whole-brain activity mapping and two-photon calcium imaging in zebrafish, we have identified a dedicated circuit for visual recognition of this kind of biological motion in a nucleus of the dorsal thalamus (DT). DT population tuning matches behavioral preference for shoal-inducing cues. Individual DT neurons encode local acceleration of visual stimuli mimicking typical fish kinetics, but are insensitive to global or continuous motion. Electron microscopic reconstruction of DT neurons reveals synaptic input from the optic tectum and projections into hypothalamic areas with conserved social function. Ablation of tectum or DT disrupts social attraction without affecting short-distance repulsion. This tecto-thalamic pathway for social recognition provides the first example of bottom-up visual social processing in a vertebrate brain.

S1.5

Collective behaviour and electrocommunication in a mormyrid weakly electric fish

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Mormyrid weakly electric fish emit electric organ discharges (EOD) for active electrolocation and electrocommunication. Self-generated signals and those emitted by conspecifics are processed via distinct sensory channels and the input from electroreceptors dedicated to communication gets centrally inhibited each time the fish emits its own EOD. During electrocommunication, identity information about the sender is encoded in the waveform of the EOD, whereas behavioral information is conveyed by modulations of the inter-discharge interval. Mormyrids can also synchronize their signals by responding to each other's EODs with very short fixed latencies, so-called echo responses. To investigate the social significance of EOD synchronizations, we used playback-emitting mobile fish dummies in experiments with single individuals and small groups of weakly electric Mormyrus rume. We found that interactive playback patterns that imitate the echo response increased the occurrence of echoing by the test fish compared to static EOD patterns. Episodes of mutual EOD synchronization were frequently associated with approaches and were mostly initiated by the approaching individual during group experiments. Since mormyrids can approach a conspecific based on the perception of that conspecifics' EODs, avoiding the refractory period within each others communication pathways by echoing with fixed latencies may allow an approaching individual to communicate social intentions, while simultaneously enabling the approached individual to acknowledge awareness of being addressed. Echoing may thus ensure the compatibility of active and passive sensing in social contexts and establish a shared social attention framework that briefly links two individuals within a group during electrocommunication.

INVITED SYMPOSIUM 2 – MECHANISMS OF ECHO– ACOUSTICALLY GUIDED NAVIGATION IN BIRDS AND MAMMALS

Different vertebrate taxa have evolved an active sensing system called echolocation. By broadcasting self-generated sounds and analyzing their echoes, which are reflected from nearby objects, echolocators can detect and classify these objects and finally navigate through structured environments. Even though echolocation behavior and its neural underpinnings have been studied for a long time, many open questions still remain. This symposium offers an overview of the progress that has been currently made towards answering the question how animals and humans find their way within 3D environments using echo-acoustic cues only. By bringing together experts who work with various model systems (birds, bats, humans) and techniques (neurophysiology, bioacoustics, psychophysics, fMRI) within the field of behavioral neurobiology, our symposium will provide a particularly integrative view on the question outlined above.

S2.1

Echolocation-specific specializations in Oilbirds

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Echolocation is an active sensing mechanism, which is based on sound production and audition. Although several taxonomic groups of echolocators exist in the animal kingdom, echolocation research shows a strong bias towards microchiropteran bats and odontocetes. To detect and classify nearby objects, echolocating animals broadcast self-generated sounds and listen to echoes of these sounds bouncing off of physical structures in the animal's environment. Especially in bats, many echolocation-specific specialization have been found. Their large and movable outer ears, for example, increase spatial directionality of hearing and thus facilitate echo detection. Bats further possess call-echo delay tuned auditory neurons in their brains, which enable them to estimate their distance towards a target. When approaching a target, both taxa, bats and odontocetes increase signal emission rates to increase the temporal resolution of their echo-acoustic sensation. We investigated the auditory system and echolocation behavior of Oilbirds (Steatornis caripensis), which represent one of the two non-mammalian groups of echolocating animals. Our preliminary data show that similar to bats and odontocetes, Oilbirds change the emission rate of their sonar signals during target approach. Furthermore, Oilbirds possess movable outer ears, which may facilitate echo detection by increasing the spatial directionality of hearing. Comparable to bats, the auditory cortex of Oilbird's seems to contain call-echo delay sensitive neurons, which may allow for target ranging. These analogies between avian and mammalian echolocation support the hypothesis that even in very distantly related animal taxa the same mechanisms are used for a behavior that evolved several times independently.

S2.2

Neural underpinnings of auditory motion tracking

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From catching a ball to navigating busy streets, many complex tasks rely on the coordination of dynamic sensory information and adaptive motor behaviors. Decades of research have been devoted to understanding visually-guided tracking, reaching, and grasping, but comparatively little work has explored auditory-guided behaviors. Our research aims to reveal the sensory mechanisms that support anticipatory auditory tracking. The superior colliculus (SC) is a midbrain structure implicated in sensorimotor integration for stimulus tracking, however, there remain gaps in our knowledge of the neural underpinnings of auditory target tracking. Here, we report on studies that exploit the scientific advantages of a mammal that specializes in auditory tracking, the echolocating big brown bat, *Eptesicus fuscus*. This insectivore produces ultrasonic vocalizations and uses features of returning echoes to track moving prey in cluttered environments. This animal's active audiomotor feedback system offers a novel model system to unravel fundamental knowledge of predictive tracking mechanisms. Here for the first time, we used Neuropixels 2.0 probes to collect large-scale simultaneous recordings from the bat SC. To simulate target occlusion and trajectory changes, we modified call-echo sequences recorded from bats tracking targets and analyzed neural responses to these stimuli. We hypothesize that neural activity persists in the SC to maintain representations of target motion through limited sensory access and contributes to relevant behavioral responses during expectation violation.

↑UP

S2.3

Do bats use echolocation for large-scale navigation?

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Navigation, the ability to plan and execute a goal-directed path, is crucial for almost all aspects of animals' life. However, how animals navigate over large-scale environments is still a riddle. Bats perform remarkable seasonal and daily navigation tasks. Nectar-feeding bats provide an example of the required high-navigation capacities of bats: they conduct seasonal migration of more than 1000 km from central Mexico to a specific cave in the Sonoran Desert. In addition, they perform daily commute flights of up to 100 km from their roost to their foraging site to feed on a particular group of preferred cacti. Another example of bats' high cognitive ability was shown in our previous study, where we showed that Fruit bats use cognitive-maps to navigate. Although recent advances in tracking technology provide us with more information about bats' behavior and their navigation capabilities, our knowledge is still sparse regarding the strategies and sensory modalities bats use to navigate, especially in comparison to birds. Here we aimed to unravel the navigation strategy of Pipistrellus kuhlii, a small-sized insectivore bat (6g), while flying in its natural environment. To examine which sensory inputs bats use to navigate, we conducted translocation experiments, manipulated the sensory information available to the bats, and allowed them to navigate using vision, magnetic sensing, smell, and echolocation. We monitored bats' movement using a reverse-GPS system (ATLAS) that provides high-resolution real-time tacking. We show that bats can perform large-scale (kilometer) navigation using echolocation and can navigate back home or to a known foraging site without the use of vision, magnetic sensing, or smell.

S2.4

Echo-acoustic behaviour and brain activity in people

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Even though every person, blind or sighted, can learn how to echolocate, to date the most skilled human echolocators are blind. The emissions that proficient echolocators prefer are mouth clicks. In our work we have found these clicks to be brief (~3ms duration) with peak frequencies 2-4kHz, but with energy also at 10kHz. The beam of these sounds spreads out in a way that is more directional than human speech, for example. We have also found that people adjust clicks dynamically and make more or louder clicks when the echo is comparably weaker. Surprisingly, when experienced echolocators localize azimuth of targets placed around them, their acuity is improved at angles that are 45° off to the side as compared to straight ahead, and this could possibly be understood in terms of binaural intensity signals. Using motion capture, we have also found that echolocation can support walking. Specifically, people who are blind and have experience in echolocation walk just as fast as people using vision, and they have walking paths that resemble those of people using vision. In brain imaging work using fMRI, it has been found that people who are blind and have experience in echolocation not only rely on auditory areas to process echo-acoustic stimuli, but that 'visual' areas are involved as well. In sighted people, early visual cortical areas are activated by visual stimulation in a specific pattern that is referred to as retinotopy. We have found that in blind echolocators, 'retinotopic' activity in primary 'visual' cortex can also be driven by sound. This result challenges our classical understanding of the organisation of human brain function by sensory modality and opens other ways of understanding the human senses.

INVITED SYMPOSIUM 3 – SENSORY INTEGRATION

Animal behavior is guided by a combination of multiple sensory cues present in the natural environment. In most cases behavior is driven not by a single but by multiple signals. Recent progress in our understanding of multisensory integration has emerged from a wide diversity of studies spanning from cellular levels to behavior, and in a wide variety of organisms, from invertebrates to vertebrates. This symposium will present advances that range from sensory integration along single command cells to integration across multiple cells in specific brain regions. The combination of techniques that allow precise subcellular mapping of activity with simultaneous recording of multiple neurons has provided this field with tools to evaluate the mechanisms by which the nervous system process salient and subtle signals from the ecosystem to extract the necessary information to carry out successfully a variety of vital functions. A more comprehensive approach that incorporates the concept of multi-signal integration and the neuronal computations that endow circuits with such properties will allow a more realistic understanding of the process that drives decision-making.

S3.1

Multisensory integration in the context of escape, from cell circuits to behavior

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Fast and accurate threat detection is critical for animal survival. Reducing perceptual ambiguity through integration of multiple sources of sensory information can enhance perception and reduce response latency. However, studies addressing the link between behavioral correlates of multisensory integration and its underlying neural basis are rare. In fish, the Mauthner cell receives inputs from the visual and auditory systems and commands the C-start escape response. We combined optic tectum and auditory stimulation with in vivo intracellular recordings to study multisensory integration in the Mauthner cell of goldfish. We found that audio-tectal cues produce a sublinear multisensory enhancement of the Mauthner cell response. In addition we found that integration in the Mauthner cells shows inverse effectiveness, i.e. that multisensory enhancement of the response is proportionally stronger for weaker cues. Paralleling electrophysiological results, behavioral experiments provided a functional role for multisensory integration. We found the strongest multisensory enhancement of the C-start when multimodal stimuli have minimum intensity while it disappears as salience increases. Temporal overlap between auditory and visual cues contribute to enhanced multisensory integration effectively decreasing response latency. That multisensory integration in single cells translates to actual behavioral advantage is a presumption that has received little empirical evidence. Here we show that Mauthner cells of goldfish integrate weak multisensory cues to enhance threat detection and reduce escape latency.

S3.2

Sensory processing in larval zebrafish: Perspectives from whole-brain calcium imaging

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Traditionally, neural activity has been monitored in great detail for one or a few cells (as in electrophysiology) or brain-wide by methods that do not provide single-cell resolution (such as functional MRI). The gap between these techniques has made it difficult to observe activity across large populations of neurons while regarding them as individual units. Because the nervous system is, ultimately, a highly interconnected network of neurons, this represents a major blind spot in our ability to describe the functioning brain. Our group is interested in the neural mechanisms by which sensory stimuli are encoded and interpreted, and in how inputs from different sensory modalities are integrated in the brain. To address the problem described above, we have adopted optogenetic and microscopic techniques that allow calcium imaging across the entire zebrafish larval brain at single-cell resolution. In the work presented here, we have applied sensory stimuli to intact, alert larvae while observing the genetically-encoded calcium indicator GCaMP6. With house-built light-sheet microscopes, we have observed large populations of neurons representing nearly the entire brain as sensory processing occurs. This work has focussed on brain-wide networks involved in vision, hearing, vestibular perception, and water flow detection, and we are now combining these modalities to study sensory integration.

↑UP

S3.3

Distributed multisensory processing systems for temporal frequency integration

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We perceive environmental oscillations by touch and audition. Recent evidence from behavioral, neurophysiological, and neuroimaging studies reveal that touch and audition are closely linked in the processing of temporal frequency information (of substrate vibrations and sound waves). Here, I summarize the recent evidence highlighting the intimate functional relationship between the somatosensory and auditory systems in temporal frequency processing. Environmental and genetic factors may play crucial roles in shaping the analogous and interactive cortical circuits dedicated to representing auditory and tactile temporal frequency signals. Interactions between audition and touch can inform the computational principles underlying multisensory integration in other sensory domains.

S3.4

Sensing scents: structural mechanism of odor recognition in insect olfactory receptors

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Animals detect and discriminate an enormous diversity of odorants by relying on the combinatorial activation of large families of olfactory receptors. A key feature of this sensory strategy is that olfactory receptors exhibit a wide array of ligand specificities, with some receptors being activated by multiple odorants and others responding to a single chemical compound. How olfactory receptors achieve this remarkable flexibility in ligand binding remains elusive, as the isolation and structural characterization of olfactory receptors from any species had not yet been achieved. In this talk I will present the first structural and mechanistic characterization of an olfactory receptor. Using cryo-electron microscopy, we elucidated the atomic structure of an olfactory receptor from an insect species, alone and in complex with two agonists—the odorant eugenol and the insect repellent DEET. These structures, together with functional insights from calcium imaging and electrophysiology, allowed us to begin defining the molecular logic of odorant specificity in insect olfactory receptors. Furthermore, this work provided the first conclusive evidence that insect olfactory receptors are the molecular targets of DEET, furthering our understanding of the molecular basis of insect repellency. These studies shed light on the basic principles that allow animals to detect a vast diversity of odorants with a limited number of receptors. In addition, it provides a structural foundation to uncover how evolution shapes olfactory receptor tuning to enable the emergence of species-specific olfactory adaptations. Finally, this research provides an inroad to the structure-based development of novel insect repellents to curb insect-borne diseases including malaria, Chagas and dengue fever.

INVITED SYMPOSIUM 4 – THE EVOLUTION OF SOUND LOCALIZATION CIRCUITS IN LAND VERTEBRATES

How did land vertebrates evolve to localize sound? It has been difficult to form coherent hypotheses about the observed variation in sound localization circuits in vertebrate auditory systems, but recently some of the confusion has been resolved in a way that focuses attention on neural coding of sound location. The key to the puzzle is that the eardrum and middle ear structures evolved independently, and from different elements, in mammalian and diapsid lineages. We discuss first what the common ancestors of terrestrial vertebrates might have heard. Second, studies of lizards, alligators and birds reveal different strategies for neural coding of sound source location. Third, evo-devo work suggests the first order auditory nuclei evolved independently in birds and mammals, resulting in a mixture of conserved, divergent and convergent features. Lastly, the different availability of binaural cues imposed distinct constraints on the “new” binaural circuits in the brainstem. We will emphasize the convergent nature of neuronal mechanisms to show how this understanding increases the explanatory power of studies of spatial processing in the vertebrate auditory system.

S4.1

Hearing with a non-tympanic ear; implications for the evolution of hearing

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Recent research has shown that tympanic middle ears evolved independently in the major vertebrate groups and thus represent independent experiments in terrestrial hearing. The tympanic ear is often regarded as a prerequisite for sensitivity to air-borne sound, but tympanic ears probably emerged late – approximately 120 my after the origin of the tetrapods and approximately 70 my after the first truly terrestrial tetrapods. Thus, the evolutionary processes leading to the functioning tympanic ear were slow and most likely gradual. Investigation of recent animals without tympanic ears show that extratympanic hearing is possible, albeit with reduced sensitivity, and that extratympanic hearing is directional. There are several instances of derived loss of a functioning middle ear, showing that in several groups, most prominently amphibians, selection pressures do not necessarily favor a tympanic middle ear. To understand the initial adaptations of the middle ear to non-tympanic hearing we will focus on the simplest mechanism for hearing – that the animal is translated, i.e., pushed and pulled, by the sound wave. This would also be the mechanism of sound interaction of an aquatic organism and is inherently directional. Models of translation by sound show that the induced vibrations depend mainly on the structure's density and its size relative to the sound wavelength. Comparison of the modelled induced vibrations with measurements of sound and vibration thresholds in lungfish, amphibians and snakes show that the sound sensitivity largely can be explained by the sensitivity to induced head vibrations, although the simple models predict higher sound sensitivity. Thus, effective transfer of vibrations to the inner ear could be an early stage of terrestrial hearing.

S4.2

Directional hearing in birds, crocodilians and lizards

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Our symposium focuses on the complex evolutionary history of directional hearing in land vertebrates. In lizards, the ears are acoustically coupled across the mouth, making them highly directional. In archosaurs (birds and crocodilians) the middle ear cavities are closed, but connected via interaural canals, resulting in less strongly coupled ears. Tympanic hearing is thought to have evolved independently in both lepidosaurs and archosaurs, but the evidence for an independent origin is not strong, and the lizard open middle ear configuration may be ancestral. Thus, comparison of these clades can reveal evolutionary changes in processing of sound direction. Both clades have very similar brainstem circuits. The auditory nerve projects to the first-order nuclei magnocellularis (NM) and angularis (NA). NA projects to the auditory midbrain, while NM projects bilaterally to nucleus laminaris (NL). Recordings from the two clades, however, show different neural coding. Free field recordings from gecko auditory nerve reveal strongly lateralized responses in each ear, forming two channels for sound source computation. Thus, the auditory nerve provides a population code for sound source direction. Archosaurs, both birds and crocodilians, compute topographic maps of auditory space in large brainstem circuits from NM to NL. These changes mark a shift from the highly directional periphery and two-channel system in lepidosaurs, to a role for coupled ears and directional neural circuits in archosaurs. Archosaurs have a well-developed ability to localize sound, and lizards should have a high capacity to differentiate between midline sound sources. Field studies of barking geckos show orientation to neighboring calls to allow for behavioral measures of sound source localization.

S4.3

A developmental perspective on the conservation, divergence and convergence of sound localization circuits

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The vestibuloacoustic nuclei of the hindbrain are the first relay point in the central nervous system for auditory and vestibular input arriving from the inner ear, via the VIIIth cranial nerve. The complement of hindbrain vestibular nuclei is highly conserved in vertebrates. In contrast, as each group of tetrapods underwent independent evolutionary processes to solve the problem of hearing on land, the hindbrain auditory nuclei acquired a remarkable mixture of conserved, divergent and convergent features. Hindbrain vestibuloacoustic nuclei develop from a highly conserved ground plan that features antero-posterior segmentation and dorso-ventral progenitor domains. As such, they provide an ideal framework on which to address the contribution of developmental processes to the evolution of neuronal circuits. We employed an electroporation strategy to unravel the contribution of two dorsoventral and four axial lineages to the development of the chick hindbrain vestibular and auditory nuclei. We compare the chick developmental map with recently established genetic fate-maps of the developing mouse hindbrain. Overall, we find considerable conservation of developmental origin for the vestibular nuclei. In contrast, the developmental origin of hindbrain auditory structures echoes the complex evolutionary history of the auditory system. In particular, we find that the developmental origin of the chick auditory interaural time difference circuit supports its emergence from an ancient vestibular network, unrelated to the analogous mammalian counterpart.

S4.4

The spatial representations of sound position in the mammalian auditory cortex

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The perception of a sound's spatial location must be computed and processed entirely within the brain, as space is not mapped onto the auditory epithelium. Interestingly, and in contrast to birds, mammals compute no topographical map of space along the early canonical auditory hierarchy. It, is, however, still debated how auditory space is neuronally represented at higher levels of the mammalian auditory system. On the one hand, the two-channel hypothesis is primarily based on auditory brainstem studies and states that in mammals, spatial information is not encoded via labeled-line projections as in the bird brainstem but rather encoded in the general firing rates of large, opposing, hemispheric channels. On the other hand, there is circumstantial evidence for cortical neurons responding to frontal locations only as well as human EEG studies postulating a third (central) channel. To investigate this apparent contradiction, we employed repeated two-photon calcium imaging in the AC of awake and anesthetized mice and probed the spatial tuning of the same hundreds of neurons over weeks. We found that evoked responses were generally stronger under awake conditions (consistent with previous studies), but also that spatial tuning toward the front of the animal was specifically suppressed under anesthesia. Spatial tuning of individual neurons changed from session to session, but the population as a whole remained predominantly stable. These findings indicate that the information from the two ears is initially contrasted in the brainstem into a two-channel system which, at the level of the cortex is then differentiated into a dynamic representation of all positions in space.

PLENARY SESSION 3

Male courtship, female visual attention, and the evolution of display complexity in jumping spiders

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Courtship displays are among nature's most exuberant expressions of biodiversity. But why are they often so complex? One possibility is that male display complexity functions to capture and retain female visual attention. Female attention is limited, and must often be split between mate assessment and other tasks, such as foraging and predator avoidance. Thus male displays may evolve to strategically direct female attention. We investigated this hypothesis using jumping spiders in the genus Habronattus, where male courtship displays involve complex movements, bright colors, and vibrational songs. We used live-interaction, video-playback, and gaze-tracking studies to better understand how male courtship traits interact with female visual attention. First, we find high levels of female distractedness during courtship, suggesting that female attention may be a limiting resource for male courtship success; in live interactions, females only spent ~27% of their time facing displaying males, whereas males faced prospective mates >99% of the time. However, male display elements such as first leg waves and third leg knee movements increase female attentiveness. In playback studies, male courtship waves are more salient and more effective than locomotory movements at capturing female attention. In live interactions, males dynamically modulate their waves: males increase their wave amplitude with increasing distance from females. When background complexity increases, males move closer to females to increase the salience of their waves. Finally, eyetracker studies indicate that male colors and movements influence where females direct the gaze of their moveable principal eyes. We discuss how female attention may have shaped male display complexity in this and other species.

PARTICIPANT SYMPOSIUM 1 – SPATIAL ORIENTATION AND NAVIGATION I

T1.1

Neural representation of goal direction in the monarch butterfly central complex

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For navigation, animals process their current heading and goal direction. This is of particular importance for migratory animals, such as the monarch butterfly that keeps its migratory direction for more than 5,000 km. During migration, sun compass information is processed in the central complex, a brain region equipped with a network of cells encoding the animal's heading direction. While heading coding has recently been investigated with neural recordings from central-complex neurons of tethered flying monarch butterflies (Beetz et al. 2022, Curr. Biol), it remains unknown how goal directions are processed in an insect brain. To shed light on this, we performed tetrode recordings from tethered flying monarch butterflies that could set a desired direction relative to a sun stimulus in a flight simulator. First, we induced changes in the heading direction of monarch butterflies by displacing the sun stimulus and observing the change in neural tuning (sun displacement experiment). As expected, the preferred firing directions of heading-direction cells followed to the butterflies' reorientation. Interestingly, the preferred firing directions of another subset of cells were unaffected by the animals' change in heading. To test if these cells may encode the goal direction, we aimed to change the butterflies' initially set goal direction without interfering with their compass coding. This was achieved by coupling the animal's initial goal direction with an aversive stimulus. After aversive conditioning, the butterflies changed their goal direction. Remarkably, the cells whose preferred firing directions were unaffected during the sun displacement experiment, were now following the animal's change in goal direction as one might expect from cells encoding for goal directions.

T1.2

Cataglyphis' magnetic compass throughout the ant's foraging career

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At the beginning of their outdoor careers, *Cataglyphis* ants perform learning walks (LWs) to acquire all information necessary for navigation as foragers later on. During the longest stopping phase of LW pirouettes, ants fixate the nest direction to memorize their way home. Since the nest entrance – a tiny hole in the ground – is invisible from their perspective, they have to rely on a reference system to guide their looks back. We could show that *Cataglyphis* novices use the geomagnetic field (GMF) as directional reference to align their gazes to the nest. Systematic alterations of the horizontal component of the magnetic field induced predictable changes in gaze directions to a new, fictive position of the nest entrance. In contrast, during their extensive outbound trips as foragers, *Cataglyphis* ants use celestial cues for path integration. Foragers perform re-learning walks (re-LWs) when the panorama at the nest entrance has been changed. Re-LWs are similar to initial LWs, but they include additional features. Furthermore, foragers do not only rely on the GMF as a reference system during re-LWs. We assume that *Cataglyphis* preferentially uses the magnetic compass information during initial LWs, and might discard it later on in favor of the celestial compass. Furthermore, we hypothesize that magnetoreception in *Cataglyphis* is an active sensing process in the ant antennae. Ants erect their antennae conspicuously during LWs. Preliminary experiments showed that *Cataglyphis* ants move their antennae differently under experimental magnetic field conditions when compared to natural geomagnetic conditions.

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T1.3

Collective navigation as a potential solution for precise navigation and homing in magnetoreceptive species

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Certain species (e.g. bogong moths, monarch butterflies) undergo long but nevertheless highly precise navigations that are at least in part mediated by sensing of the geomagnetic field. However, laboratory behavioral experiments and physical principles both suggest that the signal-to-noise ratio of the transduction of geomagnetic information may be quite low. Two solutions have previously been suggested to resolve this paradox: 1) long temporal summation of the geomagnetic signal, and 2) a switch to a different localized cue (e.g. olfactory) as the destination is approached. We examined a third possibility -- that collective navigation (i.e. flocking) may be especially useful in migrating to small locations because the efficiency of the process increases in a highly non-linear fashion as the numerical density of the animals increases as they appraoch their collective destination. Using large numbers of agent-based simulations loosely based on the North American monarch migration and that test all three methods, we found that collective navigation was dramatically more efficient at guiding individuals to precise locations, successful enough overall to explain observed migration success. We also found that the efficiency of collective navigation was strongly dependent on initial population density -- also in a non-linear fashion. Assuming that failed navigations further reduce population size, initial population loss can thus begin a runaway process leading to dramatic population decline over several migrations. Therefore if collective navigation is a factor in migration, its vulnerability to population size raises important conservation concerns in these ecologically important species.

T1.4

The honeybee's polarization compass—good dancers compensate for bad signal

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A reliable compass is the foundation of all navigation, but sources of compass information in nature are often noisy or misleading. Many animals rely on polarized skylight as part of their celestial compass system, allowing them to orient when the sun and large parts of the sky are blocked from view. The remaining skylight polarization pattern may contain information that is ambiguous or contradictory, issues that must be resolved before it can be relied upon. One straightforward solution is to integrate information across the sky, weighted [1] according to an estimate of signal reliability in each region. We present data from ongoing behavioural experiments with honeybees, designed to determine how the different qualities of polarized light affect its interpretation. In this study, we take advantage of recent technological and methodological advances that make it possible to record the properties of natural skylight[2–3], and reproduce specific parameters under laboratory conditions[2,4]. We use a novel artificial-sky stimulus to manipulate waggle dances performed by trained foragers, which are then quantified to determine how signal quality affects orientation accuracy. We find that dances retain a robust central direction even as scatter increases with greater uncertainty. Our results indicate roles for ocular anatomy and photoreceptor sensitivity in determining the weighting of polarized light cues.

1. Khaldy L, Foster JJ, *et al.* (2022) *J. Exp. Biol.* 225, jeb243734
2. Foster JJ, *et al.* (2018) *Sci. Nat.* 105(27)
3. Foster JJ, *et al.* (2021) *Curr. Biol.* 31 (17) 3935-3942.e3
4. Foster JJ, *et al.* (2019) *J. Exp. Biol.* 222, jeb.188532

T1.5

Heading memory during celestial navigation in *Drosophila*

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Many species, including humans, must know what direction to travel and remember where they are heading as they try to find food resources, mates, and hospitable habitats. In many cases, memory of a location or preferred direction is important to getting to the right place. Although Drosophila are not well-known as virtuoso navigators, field experiments revealed that they can cover 10 km of open desert in a single evening and disperse in many directions. In the lab, these flies perform straight-line orientation using a simulated sun. As a group, flies adopt a wide array of headings, but individuals have their own heading preferences and remember their headings for a few hours. However, the extent of this heading memory and factors that influence it remain unknown. Using a magnetic tether flight arena that allows the fly to rotate freely around its vertical axis, we examine the duration of heading memory in wild-type flies up to 24 hours. Individual flies are placed in a flight arena and allowed to select a preferred heading direction in response to a randomly positioned sun stimulus, while we track the body orientation in real-time. Individuals are removed from the flight arena for 6, 12, or 24 hours and then returned after the intertrial interval to test the fidelity of their heading memory. Our work tests the hypothesis that heading memory gradually decays, as flies do not require this memory to last more than a few hours during typical orientation flights. This study serves as a foundation for future work exploring factors that influence heading preference and memory, as well as the neural circuit elements responsible for this memory.

T1.6

Weighted cue integration for straight–line orientation

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Animals across a range of different species, from humans (Ernst and Banks, 2002) to insects, commonly integrate multiple sources of information to guide their behaviour, yet little attention has been devoted to describing the computation underlying cue integration. Amongst insects, ants have been observed to combine the directional information given by their path integrator with visual memories (Wystrach et al., 2015), bogong moths use the Earth's magnetic field together with landmarks (Dreyer et al., 2018), and dung beetles have been observed to utilise both sun and polarised light simultaneously during orientation (Khaldy et al., 2021). It has also been shown that the South African ball-rolling dung beetle Kheper lamarcki dynamically weights the directional input given by a sun cue and a wind cue to steer straight (Dacke et al., 2019). In this study, we go beyond previous work by characterising the reliability of an ersatz sun at different elevations and wind at different speeds as orientation cues. We also show that the relative reliability determines which cue dominates when putting their directional

information in conflict. A modelling approach further suggests that the results are best explained by continuous integration of the cues as a vector-sum, rather than a winner-take-all strategy. In addition, the neural circuitry in the insect central complex appears to provide an ideal substrate for this type of vector-sum based integration mechanism. As this integrative part of the brain appears to be highly conserved across insects, our proposed strategy might very well present us with a general solution for cue integration in insect navigation.

PARTICIPANT SYMPOSIUM 2 – MOTOR CONTROL I

T2.1

Motor adaptation is a possible explanation for light refraction correction in the archerfish

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The archerfish is unique in its ability to hunt by shooting a jet of water from its mouth at aerial targets. During a shot, the fish's mouth is in the air, while its eyes stay below the water level. Thus, in order to hit the target, it needs to overcome light refraction at the air-water interface since there is a considerable difference between the actual location of a target and the line of sight. Yet, archerfish can still hit the target with a high success rate from various angles and positions. One possible explanation for this ability is that it is learned by trial and error through a motor adaptation process. We tested this possibility by characterizing the ability of the archerfish to adapt to perturbations in the environment and to make appropriate adjustments to its shooting strategy. We introduced a perturbing airflow above the water tank with an archerfish trained to shoot at a target. For each trial shot, we measured the error: i.e., the distance between the center of the target and the center of the water jet produced by the fish. We found that archerfish can adapt to perturbation. Immediately after the introduction of the airflow perturbation, there was an increase in shot error. Then, over the course of several trials, the error decreased. After the removal of the perturbation, we observed an aftereffect: the error was in the opposite direction. Furthermore, testing the fish in two opposite airflow directions reveals that the fish adaptation is consistent with correcting for refraction and not with wind. We conclude that the archerfish is capable of motor adaptation, which is a possible explanation for the light refraction correction, as indicated by the observation that the fish was generating motor commands that anticipated the perturbing airflow.

T2.2

Birds breathe at an aerodynamical resonance

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At what rate do birds breathe? Behavior emerges from the interaction of a nervous system, a biomechanical periphery, and the environment. The nervous system, responsible for the generation of the physiological instructions driving the respiratory system, is therefore expected to be constrained by its biomechanical properties. Birds have high metabolic rates and require a continuous supply of oxygen. For that reason, it is not surprising that they developed a highly efficient respiratory system. In this work, we propose a model for the avian respiratory system and discuss how its nonlinearities constrain the emergent behavior. We fit the parameters of the model and show that normal respiration operates at its resonance. For other respiratory regimes, the nonlinearities of the system shift the frequencies at which it operates optimally. More generally, this result is consistent with an emerging picture where several life supporting devices are strikingly fine-tuned to the sources of energy that powers them.

↑UP

T2.3

Neural mechanisms of cephalopod camouflage

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Cephalopods (e.g., octopus, squid, and cuttlefish) can change their skin colors and patterns for camouflage and communication. Camouflaging cephalopods attempt to match the texture of their surrounding environment on their skin, in part by controlling the size of millions of skin pigment cells, called chromatophores, themselves under the control of a large array of motorneurons. How does the chromatophore system generate countless texture-matching patterns? Using computer-vision methods to track 105 chromatophores in behaving cuttlefish Sepia officinalis, we quantitatively described camouflage behavior at single-cell resolution. We found that the motion of camouflage patterning appears not to be pre-programed. Skin patterns move within a high-dimensional pattern space in search for a good match with the environment. As patterns "move" along these paths, chromatophores are coordinated in flexible and non-stereotypical manners. Each searching path appears to be unique and not repeated. We further identified a series of regions in pattern space, where cuttlefish tend to hold and compare their skin pattern to environment, as they progressively minimize the difference between them. These findings reveal the complexity of the chromatophore control system. To understand how the complexity has evolved, we compared Sepia with another cephalopod, the bobtail squid Euprymna berryi, which lacks complex skin patterns. We found a difference in the organization of their chromatophore lobes: a somatotopic map of motoneurons is present in Sepia but absent in Euprymna. Together our studies may start to uncover organizing principles in the neural circuits that generate a high-dimensional motor output, and may reveal how such neural circuits changed adaptively during evolution.

T2.4

How *Octopus bimaculoides* hunt with eight arms, their strategy, lateralization, and arm recruitment principles

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Octopus limb hyper-redundancy complicates our understanding of traditional motor control systems and poses the question of how decision making, and arm coordination is achieved. To investigate the principles of arm recruitment and coordination, we challenged ten Octopus bimaculoides to attack two prey items, either crab or shrimp. We used human visual classification to determine the two most reliably identifiable phases of the octopuses' visual attack: the approach and the strike, and recorded which arms were used in over six hundred prey captures. We demonstrate that visually evoked responses to prey predominantly utilized arms on the same side of the body as the eye viewing the prey, further demonstrating its reliance on monocular vision for prey capture. Moreover, we show the existence of various levels of predatory behavioral asymmetries with O. bimaculoides favoring the left visual field when it comes to crab predation while individual lateralization level was observed in shrimp predation. We found that both approach and strike significantly differed between prey types. When striking crabs, the synchronous recruitment of several arms is preferred while for shrimp, a sequential arm recruitment with a characteristic swaying movement is preferred. In both cases, these motor programs were highly stereotypical and involved mostly adjacent arms supporting a dimension reduction approach to decrease the complexity required for motion control of hyper redundant limbs. These findings demonstrate that octopuses adjust their predation strategy to prey type and use simple peripheral motor programs for predatory strike to enable efficient coordination of their eight arms.

T2.5

Neural organization of jaw movements in fish

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The evolution of jaws in ancient fishes was integral to the subsequent radiation of vertebrates, serving as a substrate for the emergence of novel behaviors, including the diversification of feeding strategies. However, little is known about how motor circuits adapted to control orofacial movements during jaw evolution. Here, we identify a premotor network that controls predatory feeding strikes in fish. Performing two-photon calcium imaging in semi-restrained zebrafish larvae during feeding strikes revealed circuits distributed throughout the reticular formation underlying this behavior. Central to this network are T-interneurons, a heterogeneous neuronal population in the reticular formation, which have previously been shown to coordinate premotor and motor centers during escape behavior. We found that optogenetic stimulation of T-interneurons drove fast, stereotyped orofacial movements resembling feeding strikes. Neuroanatomical and synaptic tracing in a dense electron microscopy reconstruction demonstrated that each T-interneuron contacts motor neuron pools distributed over multiple nuclei in the brainstem and spinal cord that control a unique subset of muscles involved in feeding. Together, these findings suggest a modular encoding of orofacial movements in the reticular formation. Our results suggest a broad role for T-interneurons in integrating orofacial movements with other body movements across a range of behaviors, including both feeding and escape. Such a circuit may be conserved across taxa given the single ancient origin of jaws shared by all extant jawed vertebrates.

T2.6

Sensorimotor transformation in the brain of *C.elegans*

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The problem of sensorimotor transformation and the origin of trial-to-trial variability in behavior are fundamental problems in neuroscience. We used the nematode worm *C. elegans* as a model organism to provide new insights into this question. As a suitable experimental paradigm, we focused on oxygen sensory circuits, which control locomotion responses to changes in environmental oxygen concentration. Single cell resolution real-time whole-brain calcium imaging recordings in immobilized animals were used to access the activity of most neurons in the brain while the animal was stimulated with a periodic oxygen stimulus. By measuring motor circuit activity we validated that this paradigm leads to a successful fictive motor response. While we observed reliable responses in oxygen sensory circuits, motor circuits responded in a probabilistic fashion exhibiting substantial trial-to-trial variability, reminiscent to the variability observed in behavioural experiments. First, we showed that internal state of motor command interneurons and past history of their activity influence responsiveness of the system and accounts for part of the response variability. Next, we developed a data mining approach, via which we could systematically disentangle between neurons with sensory and motor related activities. Moreover, we revealed several interneuron candidates, the activity profiles of which could predict the instantaneous motor outcome. In summary, our approach revealed sources of the behavioural variability origins across a whole brain and offers opportunities for future studies investigating the underlying mechanisms.

PARTICIPANT SYMPOSIUM 3 – LEARNING, MEMORY AND COGNITION I

T3.1

The impact of brain lateralization on quantity discrimination abilities in zebrafish (*Danio rerio*)

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Brain lateralization refers to structural and/or functional differences between the left and the right hemispheres. Once thought be unique to humans, functional specialization has proven to be widespread among non-human animals. There is increasing evidence that atypical brain lateralization is associated with neuropsychiatric and cognitive disorders. The ubiquity of lateralized brain functions has allowed the establishment of animal models to investigate brain lateralization as a determinant of behavioural function and dysfunction. As zebrafish display lateralized behaviours related to neuroanatomical asymmetries in the epithalamus, they are ideal to explore how brain asymmetry is related to brain functions. Here we investigated the impact of brain lateralization on quantity discrimination abilities in zebrafish with different patterns of brain asymmetry. To this aim, *southpaw* morpholino injected larvae with left parapineal, right parapineal, and middle parapineal and *sox1a*$^{-/-}$ mutants with double-right dorsal habenula phenotype were tested in a spontaneous choice test. We assessed fish ability to discriminate between numerically different shoals of conspecifics (1 vs. 3, 2 vs. 3 and 2 vs. 5). We also assessed the asymmetric distribution of pallial-expressed genes (*grik1a, robo1, eomesa, arrb2, gap43*) in zebrafish as a function of the laterality phenotypes. Results showed that disruption of Left-Right epithalamic asymmetry impacted on gene expressions and discrimination abilities thus providing evidence of a relationship between atypical patterns of brain lateralization and quantity estimation.

T3.2

An insect brain organizes numbers on a left–to–right mental number line

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The ‘mental number line’ (MNL) is a form of spatial numeric representation that associates small and large numbers with the left and right space, respectively. This spatial-numerical organization can be found in adult humans and has been related to cultural factors such as writing and reading habits. Yet, both human newborns and birds order numbers consistently with a mental number line, thus questioning culture as a main explanation for MNL. Here we explored the numeric sense of honey bees and show that after being trained to associate numbers with sucrose reward, they order novel numbers from left to right according to their magnitude. Importantly, the location of a number on that scale varies with the reference number previously trained and does not depend on low-level cues present on numeric stimuli. We provide a series of neural explanations for this effect based on the extensive knowledge accumulated on the neural underpinnings of visual processing in honey bees. We conclude that the MNL is a form of numeric representation that is evolutionary conserved across nervous systems endowed with a sense of number, irrespective of their neural complexity, and probably related to existing brain asymmetries.

T3.3

Neuron counts reveal the evolution of brain complexity in land vertebrates

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The evolution of brain processing capacity has traditionally been inferred from data on brain size. However, similarly sized brains of distantly related species can differ in the number and distribution of neurons, their basic computational units. To get a more reliable picture of the evolutionary paths leading to high cognitive power, we have analyzed neuron numbers in more than 350 species of non-mammalian tetrapods, combined this with previously published data on mammals and used this unique dataset to reconstruct for the first time the evolution of brains across tetrapods by directly analyzing neuron numbers. Amphibians have not only small brains but also low neuronal densities and remarkably low absolute neuron numbers. However, frogs have much higher neuronal densities than caudates, comparable to non-avian reptiles. Likewise, neuron densities and numbers are modest in reptiles, ranging from millions to tens of millions. Birds and mammals have dramatically increased neuron numbers in the telencephalon and cerebellum, brain parts associated with higher cognition. They have on average about 17- and 9-fold more neurons, respectively, in the telencephalon and about 45- and 69-fold more neurons in the cerebellum than expected for reptiles of equivalent body mass. Two other major increases in the number of neurons happened in groups known for their cognitive prowess – core landbirds and anthropoid primates. Importantly, phylogenetic analysis suggests that only a handful of major changes in neuron-brain scaling in over 350 million years of evolution pave the way to intelligence in land vertebrates.

T3.4

Learning with a decentralized nervous system in the brittle star *Ophiocoma echinate*

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Like other members of Phylum Echinodermata, ophiuroids are marine invertebrates that lack centralized nervous systems. They have five radially arranged ganglia joined by a central nerve ring. While operant and classical conditioning have been demonstrated in sea stars (Class Asteroidea) in a limited number of studies, members of the other echinoderm classes remain relatively untested. Here, we tested whether individuals of the ophiuroid species Ophiocoma echinata were able to learn an association between a period of darkness and the presentation of a food reward. An experimental group was trained by presenting food during a period of darkness, while control group animals were fed under regular daytime room lights many hours after the period of darkness. After the training period, the experimental group demonstrated they had learned to associate the two cues by regularly emerging during the dark period even when no food was presented. The untrained control animals, as well as pre-training experimental animals, did not emerge during the dark periods when no food was presented. This study shows that classical conditioning is possible in a new class of animals that lack centralized nervous systems.

T3.5

First evidence of advanced learning in jellyfish

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Advanced learning, such as classical or operant conditioning, has never been unequivocally associated with animals outside vertebrates, arthropods, or molluscs. Learning modulates behaviour and is imperative for survival in the vast majority of animals. Obstacle avoidance is one of several visually guided behaviours in the box jellyfish, *Tripedalia cystophora* Conant, 1897 (Cnidaria: Cubozoa) and intimately associated with foraging between prop roots in their mangrove habitat. The obstacle avoidance behaviour is a species-specific defence response (SSDR) for *T. cystophora* and identifying such SSDR is essential for testing the learning capacity of a given animal. Using the obstacle avoidance behaviour we are able to show that box jellyfish, animals without a conventional brain, performed advanced learning (operant conditioning). Further, we found that the rhopalial nervous system is the learning centre and that *T. cystophora* combines visual and mechanical stimuli during operant conditioning. Since *T. cystophora* has a dispersed central nervous system lacking a conventional centralized brain, our work challenges the notion that advanced learning requires complex neuronal circuitry. It suggests the intriguing possibility that advanced neuronal processes, like operant conditioning, is a fundamental property of all nervous systems.

↑UP

T3.6

Learning and innate predispositions contribute to variation in songbird introductory gestures

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Animals communicate with each other using complex displays and many of these displays begin with the repetition of an introductory gesture. For example, Anolis lizards begin their territorial displays with a variable number of push-ups, and songbirds produce a variable number of introductory notes (INs) before their courtship songs. In the zebra finch, a well-studied Australian songbird, the number and structure of INs varies considerably across individuals. Whether this variation is a result of learning, similar to the learning of song, remains poorly understood. Using a combination of tutoring methods, we found that both the number and acoustic structure of INs are learned from a tutor. The accuracy of copying IN number was independent of the accuracy of copying IN structure. Interestingly, zebra finches that were not exposed to a tutor, also produced INs before their song, and these INs shared many similarities with INs in normally reared birds. Currently, we are using lesion techniques to identify the neural pathways involved in the learning of IN number and IN structure. Overall, these results demonstrate that INs and variation in IN number and acoustic structure, are shaped by both learning and innate predispositions. More generally, our results suggest that innate predispositions maintain the species-specific structure of the entire display while learning allows for similarities within small groups (nestmates in this case).

PARTICIPANT SYMPOSIUM 4 – VISUAL SYSTEM I

T4.1

To see with multiple rhodopsins: extraordinary vision in the deep-sea fishes

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In the dim light habitat, such as deep sea, fish often obtain visual information by light-sensitive photopigments based on a single rod opsin (rhodopsin 1 = RH1). Vast majority of teleost fishes possess one or two copies of the rhodopsin gene in their genome. In some deep-sea fishes, however, extreme adaptations of rhodopsins have been found. We have recently discovered a species, silver spinyfin (Diretmus argenteus), with the highest number of visual opsins among vertebrates (two cone opsins and 38 rod opsins). Such an extraordinary set up has convergently evolved also in two other unrelated deep-sea lineages (lanternfishes and tube-eye fish), and it suggests unique mode of vision in the extreme conditions. The peak sensitivity of the spinyfin rhodopsin photoreceptors spans the blue-green light spectrum of 444 to 519 nm, which is actually one of the shortest-sensitive rhodopsins known in vertebrates. Such extreme diversity of the rhodopsin genes has raised via multiple gene duplications followed by a series of mutations causing changes of the protein function (=sensitivity). Following the original discovery, we focus on genomic organisation of the RH1 genes and we have expanded the retina transcriptome data set from the recently collected deep-sea samples. As the last point, we found differential function of vision during development when larvae need to see well in the shallow, while adults in the deep. We found that rhodopsins are the most important vision genes in the depth, whereas the deep-sea fish larvae rely broadly on the green-sensitive (RH2) cone opsin gene and it seems to be conserved across teleosts. Spinyfins take advantage on having multiple rhodopsin genes and they complement the cone opsin by several “larval” rhodopsins during their transition phase.

↑UP

T4.2

Plasticity of coral reef fish vision in a changing world

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Coral reef fishes inherently experience significant changes in their light environment as conditions on the reef fluctuate with depth, season, and increasingly also due to human-induced sediment suspension or algal blooms. Recent research indicates that some reef fishes may be able to adjust their visual systems to accommodate such changes in light habitat. If coral reef fishes could 'pick and choose' their visual system according to the prevailing light conditions, it may make them more resilient and enhance their survival. Here, I will present the results from two of our recent studies investigating the plasticity of coral reef fish vision. First, we will explore visual gene (opsin) expression and changes thereof across seasons in two typical coral fish families, the damselfishes (Pomacentridae) and the surgeonfishes (Acanthuridae). As the light environment on the reef shifts from a broad-spectrum clear body of water during winter to a more red-shifted algal dominated habitat in summer, the opsin gene expression of some but not all species seems to track these changes. Next, we will be looking at short-term plasticity in developing and adult Convict surgeons, Acanthurus triostegus. Exposing the fishes to various light environments, including artificial light at night (human light pollution) for as little as six days, revealed more pronounced plasticity in extreme environments such as constant darkness. Developing fishes also showed a stronger response compared to adults. Overall, our results show the potential for both long- and short-term plasticity on the reef, with development, ecology and phylogenetic inertia affecting the strength of plasticity found within and between species.

T4.3

Neural basis of object recognition in the visual system of the archerfish

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Recognition of individual objects and their categorization into groups is a complex computational task. Nevertheless, the visual system is able to perform this task rapidly and accurately. Animals can efficiently recognize objects despite countless variations in the image projection on the retina that constantly change due to different viewing angles, distances, light conditions, and other parameters. To better understand the recognition process in the teleost, we explored it in archerfish. Archerfish hunt by shooting a jet of water at aerial targets. This unique trait allows us to monitor the fish's visual decisions by presenting it with a set of images on a screen above the water tank and observing the behavioral response. By manipulating various features of the presented objects, we study their contribution to the fish decision-making. To further understand the underlying computation, we developed a computational model based on object features and a machine learning classifier. Analysis of the model revealed that a small number of features was sufficient for categorization, and the fish were more sensitive to object contours than textures. To understand the neural basis of object recognition, we looked into the neural representation of features and the objects from two different categories in the archerfish brain using an electrophysiological recording of single cells in the optic tectum. Our findings suggest the existence of an elaborated representation module in the archerfish visual system that allows the extraction of relevant features of an object and its categorization.

T4.4

Brain-wide visual habituation networks and escape responses to looming stimuli, and the effects of saliency, timing, luminance and motion

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Animals from insects to humans perform visual escape behavior in response to looming stimuli, and these responses habituate if looms are presented repeatedly without consequence. Due to technical challenges, the brain-wide networks mediating loom habituation, and how differences in the stimulus properties modulate these habitation networks are poorly understood. Here, we present a combination of experiments where we performed both behavioral analyses and brain-wide cellular-resolution calcium imaging in larval zebrafish while presenting them with visual loom stimuli of different features, or stimuli that selectively deliver either the movement or the dimming properties of full loom stimuli. The first set of experiments show that distinct functional categories of loom-sensitive neurons are located in characteristic locations throughout the brain, and that both the functional properties of their networks and the resulting behavior can be modulated by the stimulus saliency and timing. With another set of experiments we then find that moving edges or the drop in luminance components have a different effect on the escape responses, and that they don't habituate responses to a subsequent full loom stimuli, suggesting that full looms are required for habituation. Our calcium imaging reveals that motion-sensitive neurons are more abundant that dim-sensitive neurons, and that neurons responsive to both stimuli (and to full loom stimuli) are concentrated in the tectum. Overall, our results suggest that different populations of neurons and their circuits can habituate at different rates, and that, while both movement- and dim-sensitive neurons contribute to predator escape behavior, neither plays a specific role in brain-wide visual habituation.

T4.5

A map of trematode worm infection of the dragonfly (*Sympetrum sp*.) brain: Anatomical evidence for parasite control of behavior

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Parasitic infections can influence the behavior of the host to facilitate their life cycle. Insects are intermediate hosts for many parasites, which modify the insect's behavior to increase the likelihood they will be eaten by the final host of the parasite. Adult dragonflies serve this role for trematode worms. The worms form cysts in a dragonfly's brain, and subsequently the dragonfly fails to escape from bird or amphibian predators. Here, we ask whether the location of cysts in the brain can explain the parasite's influence. We have created a preliminary atlas of the uninfected brain of dragonflies in the genus Sympetrum, using common immunofluorescence methods and confocal microscopy. We then image infected brains in situ using micro-computed tomography (micro-CT) with iodine as a contrast agent, and map the location of the cysts. Cysts were predominantly located in: the optic lobes, especially between the medulla and the lobulla; the periesophageal, ventromedial, and lateral complex neuropils on the ventral surface of the brain; and the superior neuropils on the rostral surface. Cysts were large relative to the volume of the neuropil, and we observed evidence of significant damage to the neuropils. Our results suggest that trematode worm infection impacts the visual system, visual navigation, and descending motor control in the dragonfly

T4.6

Visual system and its developmental changes in European cyprinid fishes (family *Cyprinidae*)

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Vision plays a key role in life history of fish, and is mediated by photoreceptor cells (rods and cones) in retina. Teleost fish possess a set of opsin genes in their genome encoding four types of photosensitive proteins for colour perception (SWS1, SWS2, RH2 and LWS on cones) and one type of opsin for dim-light vision (RH1 on rods). During evolution, some fish acquired multiple copies of opsin genes, and they use (=express) a subset of those in rods and cones, each opsin responsive to a specific wavelength of visible light spectrum. In this study, we focused on 21 species of cyprinid fishes, majority of them from subfamily Leuciscinae, living in Central Europe. We sequenced retinal transcriptomes and identified the opsin genes participating in colour perception, further confirmed by genomic data. Cyprinids express four types of cone opsins, some of these in multiple copies. We report the plasticity of visual system comparing data of adults and juveniles of 11 species. In adults, the most abundant opsin in retina is the long wavelength sensitive LWS opsin, which may be a result of adaptation to a higher level of water turbidity in European rivers. Larvae and juveniles, however, predominantly express shorter wavelength-sensitive opsins (SWS1, SWS2) as they linger close to the surface on shallow riverbanks. This pattern may also represent a developmental constraint shared among teleost fishes. Furthermore, we employed FISH in-situ to visualize the distribution of single- and double cones in the photoreceptor mosaic in the cyprinid retina.

FRANZ HUBER LECTURE

The four Fs of studying neural circuits underlying behavior: form, function, phylogeny, and fortune

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Although neuroscience tends to emphasize the relationship between form and function in the study of the neural basis of behavior there are two other factors that play important roles: phylogeny and fortune. The proximate mechanisms by which neural circuits function are related to their form. That is, the structural components of neurons and synapses comprise the machinery that underlies the generation of behavior. However, the ultimate explanations for why neurons and circuits have those forms are rooted in their phylogeny. Fortune comes into the picture because all unexpected discoveries arise from chance. I will talk about the neural circuits underlying the swimming behaviors in nudibranch molluscs. These central pattern generator (CPG) circuits are composed of large individually identified neurons. In one species, the swim CPG contains only four neurons. We understand the precise mechanism by which these neurons generate a simple motor pattern. A different mechanism is used by the same neurons in another species to produce the same behavior. To gain a deeper understanding of how neurons gain their identities and their species-typical connectivity, my lab switched to studying a nudibranch species that could be raised in the lab. I will talk about how our efforts to map all of the neurons and their gene expression patterns led to a stroke of good fortune, which diverted us from the small number of large neurons to the large number of small neurons that make up the so-called “peripheral” nervous system. We found that there are far more neurons in peripheral ganglia than in what we considered to be the brain. We discovered a great deal of interconnectivity between sensory systems. Fortunately, this is leading us to form new ideas about how this phylum functions.

YOUNG INVESTIGATOR AWARDS

Y.1

Blink and you'll miss it: Ballistic predatory behavior in the ogre–faced spider

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Nocturnal animals, who live in a world dramatically darker than their diurnal relatives, typically possess sensory systems attuned to their dimly lit lifestyle. The ogre-faced spider, Deinopis spinosa, is no exception. These spiders live a circadian Jekyll and Hyde lifestyle, avoiding predators by day, through total immobility and camouflage, and stealthily ambushing prey by night. The sensory systems of this predator are well-adapted for nocturnal foraging - massive, motion-sensing eyes, complemented by multiple sensory organs used to detect airborne acoustic information. These sensory systems allow D. spinosa to exhibit a unique style of active foraging, termed "net-casting." While suspending themselves above the ground in a frame web, these spiders hold a specially made net in their front four legs and wait for insects to pass by. The near approach of prey triggers explosive acts of body movement and net manipulation that underlie an uncanny ability to ensnare prey walking beneath (forward prey strike) or flying above (backward prey strike). Through a combination of behavioral and neurophysiological studies, we show sensory modality partitioning in net-casting behavior, dependent on prey type (i.e. cursorial vs. aerial). Here, I will present our insights into the sensory ecology of net-casting, highlighting the capabilities and limitations of vision and hearing as they pertain to foraging in this fascinating group of animals.

Y.2

Bone conduction of sound supports aerial hearing and directional sensitivity in salamanders

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Inherently directional otolithic ears evolved early in vertebrate evolutionary history, revealing the significance of sound detection and localization to survival; however, fossil evidence indicates that the aquatically-adapted ear of early terrestrial tetrapods lacked specializations for transducing aerial sound pressure (e.g., tympana) and therefore required extratympanic mechanisms for hearing. We investigated hearing in atympanic salamanders to evaluate pathways for sound transmission to the inner ear in the absence of a tympanic middle ear. We used auditory brainstem response (ABR) recordings to measure sensitivity to sound pressure and seismic vibration stimuli. ABR measurements combined with laser Doppler vibrometry showed that aerial hearing in salamanders is mediated by the detection of skull vibrations that are generated by the impinging sound pressure waves. We further observed that these sound-induced skull vibrations vary with incident angle of the sound. Comparison of directional ABRs with vibration velocity measurements in salamanders reveal that bone conduction hearing supports a figure-eight pattern of directional sensitivity to airborne sound in the absence of a pressure-transducing tympanic ear.

Y.3

Numerical discrimination in *Drosophila melanogaster*

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Sensitivity to numbers is a crucial cognitive ability that is widespread across the animal kingdom. The lack of experimental models amenable to systematic genetic and neural manipulation has precluded discovering neural circuits required for numerical cognition. Here, we demonstrate that, when provided with a choice, Drosophila flies spontaneously prefer sets containing larger numbers of objects. This preference is determined by the ratio between the two numerical quantities tested, a characteristic signature of numerical cognition across species. Individual flies maintained their numerical choice over consecutive days. Using a numerical visual conditioning paradigm, we found that flies are capable of associating sucrose with numerical quantities and can be trained to reverse their spontaneous preference for large quantities. Finally, we show that silencing LC11 neurons reduces the preference for more objects, thus identifying a neuronal substrate for numerical cognition in invertebrates. This discovery paves the way for the systematic analysis of the behavioral and neural mechanisms underlying the evolutionary conserved sensitivity to numerosity.

↑UP

Y.4

Specialized mechanosensors in flying insects

Alexandra Yarger[1,2]; Zehao Li[1]; Janka Kluge[1]; Igor Siwanowicz[3]; Huai-Ti Lin[1]; Jessica Fox[2]
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The specificity of sensory systems for behavior is essential for survival, particularly during challenging conditions. For mechanosensation, flight is such a condition, where the animal is bombarded with aerodynamic and inertial forces. In dipteran insects (flies), modified hindwings called halteres detect forces produced by body rotations and are essential for flight. Mechanosensory information from the halteres is sent to neck and wing-steering motoneurons, allowing direct control of gaze and body attitude. Using intracellular electrodes to record haltere primary afferents, I investigated the mechanisms by which haltere neurons transform inertial forces into patterns of neural spikes and propose a mechanism by which single neurons can encode three-dimensional haltere movements during flight. Odonates (dragonflies and damselflies) are also known for their complex flight behaviors. Unlike flies, they do not possess halteres to detect inertial forces and instead rely on their highly sensitive wings for both aerodynamic and inertial sensing. To understand the aeroelastic wing deformations resulting from external forces, we computationally extracted dominant features of wing motion. I then recorded extracellularly from wing afferents in response to airflow and implemented neural models to determine whether these features accurately predict neural activity. The fly and the dragonfly provide a unique contrast in the contexts of evolution, ecology, biomechanics, neurobiology, and behavior. By using comparative approaches to explore the unique sensory needs of flying insects such as these, we will expand our understanding of how complex and specialized behaviors have arisen.

WEDNESDAY, 27 JULY 2022

PLENARY SESSION 4

The neural basis of host seeking in skin-penetrating parasitic nematodes

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Skin-penetrating parasitic nematodes infect over a billion people worldwide and are a major cause of neglected tropical disease. These parasites have an infective larval stage that actively searches for hosts using host-emitted sensory cues, then infects by penetrating through the skin. We are investigating the host-seeking and host-invasion behaviors of these parasites, as well as the molecular and neural mechanisms that underlie them. We use the human-parasitic threadworm *Strongyloides stercoralis* for these studies because *S. stercoralis* is unique among parasitic nematodes in its amenability to genetic manipulation. We found that *S. stercoralis* infective larvae are robustly attracted to a diverse array of human-emitted sensory cues, including body heat. CRISPR/Cas9-mediated targeted mutagenesis of the *S. stercoralis* homolog of the *C. elegans* cGMP-gated cation channel subunit gene tax-4 is required for heat seeking, indicating that conserved molecular pathways mediate host seeking in *S. stercoralis* and environmental navigation in *C. elegans*. We then identified the primary thermosensory neurons of *S. stercoralis* and found that they use a fundamentally different temperature-encoding strategy than C. elegans thermosensory neurons. We further showed that *S. stercoralis* has three thermoreceptor proteins, one tuned to temperatures ranging from ambient to host body heat and two tuned to temperatures near host body heat. Our results demonstrate that parasite-specific behaviors such as heat seeking arise from functional adaptations at the earliest stages of sensory processing. We are now using a similar approach to study skin-penetration behavior. In the long run, our results may enable the development of novel nematode control strategies.

INVITED SYMPOSIUM 5 – MEMORIAL SYMPOSIUM IN HONOR OF BARRIE FROST AND JACK PETTIGREW, LEADERS IN THE FIELD OF NEUROETHOLOGY

Sensation and orientation are two key features of animal behavior. This symposium shall honor two colleagues who left big footsteps in our understanding of the neuro-ethology of these two tasks. Both, the late Barrie Frost and the late Jack Pettigrew published landmark work on sensory processing. Seminal insight into stereo vision and looming in model organisms like the cat and the pigeon is tied to their career. Likewise, the two were always attracted by more exotic living beings like the monarch butterfly, the Australian bogong moth, the tengmalm's owl, plathypus, fruit bats, frogmouths, and Tibetian monks to name but a few. The symposium tries to cover aspects of the research of these two colleagues that up to today persist as hot topics in 4 talks. Henrik Mouritsen (Oldenburg, Germany) will talk about navigational strategies in birds, specifically their magnetic compass, while Eric Warrant (Lund Sweden) will report on the latest findings on the mechanism underlying long-distance navigation in the Australian bogong moth. On the other hand, Thomas Cronin (Baltimore, USA) will highlight eye-movement strategies in birds and mantis shrimps. Finally, Justin Marshall (Brisbane, Australia) will talk about the inspiring enthusiasm and his extraordinary ideas that influenced the careers of many scientists. In this way Jack also sparked research beyond current model systems and pioneered how such research may uncover effective adaptations acquired through selection pressures in evolution. Understanding such solutions from a mechanistic point of view offers opportunities for biomimetic and translational applications.

↑UP

S5.1

How the stars and the Earth's magnetic field guide the long migratory journey of an Australian moth – a tribute to Professor Barrie Frost

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Nocturnal migratory birds are famous for their remarkable ability to migrate many thousands of kilometres, from one specific place on the surface of the earth to another. In recent years, researchers have discovered that migratory birds steer their journey in the right direction with the help of an (as yet unknown) magnetic sense which utilizes the earth's magnetic field as a compass. Moreover, researchers have also discovered that these birds have the possibility to use the starry sky as an additional compass. Using a simple but remarkable device invented by my late friend Barrie Frost, we discovered that an Australian migratory moth - the nocturnal Bogong moth Agrotis infusa - has exactly the same abilities, despite possessing extremely small eyes and a brain smaller than a grain of rice. During my presentation I will describe the Bogong moth's natural history, and how Barrie and I made our first discoveries of its remarkable navigational abilities. Barrie's enthusiasm, curiosity, creativity, boundless good humour and generous spirit lifted all of us, and our times in the Australian bush are just not the same without him. As Barrie continuously reminded me, science is the most fun when done with friends. How right he was.

S5.2

Quantum birds: The magnetic compass sense of night-migratory songbirds

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In my presentation, I will present how night-migratory songbirds seem to use a quantum mechanical mechanism to sense magnetic compass information which they use to navigate with exquisite precision over thousands of kilometres (Mouritsen 2018). In the past years, evidence has mounted that migratory birds use a light-dependent, radical pair-based mechanism to sense the axis of the geomagnetic field lines (Hore & Mouritsen 2016). The magnetic compass of night-migratory birds is sensitive to anthropogenic electromagnetic field disturbances being ca. 1000 times weaker than the current WHO guideline limits (Engels et al. 2014; Schwarze et al. 2016). This result strongly indicates that the basic sensory mechanism underlying the magnetic compass of night-migratory songbirds should be based on quantum mechanical principles rather than classical physics. Neuroanatomical data have shown that magnetic compass information is detected in the eye and then processed in a small part of the thalamofugal visual pathway terminating in the visual processing centre "Cluster N" (Mouritsen et al. 2005; Liedvogel et al. 2007; Feenders et al. 2008; Zapka et al. 2009). When Cluster N is deactivated, migratory European Robins can no longer use their magnetic compass, whereas their star compass and sun compass abilities are unaffected (Zapka et al. 2009). The lagena and associated pathways remained intact. Bilateral section of the trigeminal nerve had no effect on the birds' ability to use their magnetic compass (Zapka et al. 2009). Very recently, we could also show that the light-sensitive protein Cryptochrome 4 from a night-migratory songbird is magnetically sensitive *in vitro* based on a radical-pair mechanism (Xu et al. 2021).

S5.3

Remembering Barrie Frost and Jack Pettigrew: Eye movements in birds and in the weird, typically Australian creatures, mantis shrimps

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As different as they were individually, Barrie Frost and Jack Pettigrew both were respected widely and affectionately throughout the research community of comparative neurobiologists. In the research of my own laboratory, I was inspired by Barrie's work on head and gaze stabilization in birds. His studies of the short-necked domestic pigeon somehow seemed relevant to head movements in tall, long-necked cranes and led to my laboratory's work on head stabilization in walking and foraging whooping and sandhill cranes. These animals adjust their walking speed to allow them to stabilize gaze for about half the time, and probably use the head's forward projection to foster active vision via optic flow as well. In contrast, my work with Australian animals, much of which was done at the University of Queensland's Vision, Touch, and Hearing Research Centre established by Jack in the late 1980's, included research on the very different eye movement strategies of the odd marine crustaceans known as mantis shrimps. Jack was a great booster of work on Australian creatures and was always supportive of my vision sciences effort "down under". I consider myself fortunate to have been in the company of two of such inspiring neuroscientists as Barrie and Jack. Their work leaves a legacy that will always be part of research that considers how animals sense and move, and how they experience the external world and interact with it.

S5.4

Jack Pettigrew – the secret to a successful scientific career

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Somebody take the wheel!" Jack cried, leaping from the speeding speed-boat he was driving along the Great Barrier Reef to join dolphins in the water. Looking at Jack skipping across the water, more like a stone than an eminent professor, I was yet again amazed by this man's lust for life and love of the natural world. As the Australian expression goes, he loved "getting amongst it". His love of nature made him many things including an explorer and mountain climber (the first up Ball's Pyramid), bird-twitcher with an encyclopaedic-brain for all things birdie, teacher of Australian bush-lore and of course a superb neuroethologist. I was fortunate to join the Vision Touch and Hearing Research Centre towards the end of its run as Australia's first federally funded centre of excellence. Putting the VTHRC together was another of Jack's passions and he collected together many brilliant and up-and-coming scientists (and me) to form this pump-house of Nature and Science publications. Jack was many things, including brilliant, enthusiastic, sometimes painful and provocative, but a great mentor to young scientists, allowing them to flourish and grow in the unique environment he created. I will present a few of the flowers I grew in his garden, including more mysteries from mantis shrimps and the truth about fluorescence, but will also reflect on the many mountains of knowledge and discovery Jack climbed on his way to the top.

INVITED SYMPOSIUM 6 – OVERLOOKED FOR DECADES? MOTONEURON INVOLVEMENT IN RHYTHM GENERATION

Motoneurons are traditionally considered the last relay from the central nervous system to muscle control in a given motor behavior. Rhythm-generating circuits, Central Pattern Generators (CPGs), send projections to motoneurons, which in turn project to and generate appropriate muscle contractions. However, in several species and motor systems, there is evidence accumulating that motoneurons play a more complex role in pattern generation itself. These studies have changed the existing dogma of motoneurons being only a relay station between the CNS and the periphery, and have shown that motoneurons directly influence the circuits responsible for pattern generation. Motoneurons can influence premotor circuits, via axon collaterals or electrical coupling, to modulate premotor circuit activity. Studies of central circuits in highly diverged species – including Drosophila, C. elegans, leeches, crustaceans, rodents, fish, and frogs – have indicated a crucial role of motoneuron feedback in maintaining normal behavior patterns dictated by central pattern generator activity. In this symposium, talks will explore current studies examining the role of motoneuron feedback activity across many different taxa and behaviors, and will examine how widespread motoneuron participation in motor circuits may be. The broad diversity of animal models in this symposium will highlight the importance of motor-premotor neuron interactions in patterning of motor activity across animals, which will encourage the audience to consider potential divergence and convergence of motor circuits across the animal phylogeny.

S6.1

Exploring the role of motor feedback in vocal evolution

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How do behaviors evolve? One way to address this question is to identify differences in neural circuits that generate distinct behaviors in closely-related species. In order to identify neuronal mechanisms underlying vocal evolution, we investigated a hindbrain circuit that controls vocal patterns in two frog species that diverged ~17 mya, *Xenopus borealis* and *X. laevis*. Males of both species produce advertisement calls to attract mates, however their temporal structures differ: *X. laevis* calls consist of many rapid (~60 Hz) sound pulses, while each *X. borealis* call consists of a single sound pulse. To understand the physiological mechanisms underlying these patterns, we used extracellular and whole-cell recordings in fictively calling brains, which produce vocal nerve compound action potentials (CAPs) corresponding to sound pulses in species-specific calls. In *X. laevis*, CAPs are brief (~5 ms), due to highly synchronous activation of vocal motoneurons that, in turn, receive excitatory ~60 Hz input from a group of premotor neurons coding fast rate calls. Each CAP is driven by 1-2 precisely timed premotor spikes. The 60 Hz firing frequency of these premotor neurons depends on a feedback signal from the motor nucleus. In contrast, X. borealis CAPs are long (~50 ms), due instead to asynchronous motoneuron activation, driven by bursts of rapid premotor neuron spikes. We compare the vocal circuits underlying the advertisement call patterns of both species to explore whether differences in the motoneuron feedback pathway in X. borealis compared to *X. laevis* contribute to their distinct motor output patterns. This research should uncover the importance of motoneuron feedback in producing motor output, as well as whether it can play a role in the evolution of unique behaviors.

S6.2

The involvement of motoneurons in the patterning of spinal locomotor patterns in zebrafish

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The execution of movements entails precise control of motor neuron activity. The prevailing view in motor control, including locomotion, considers motor neurons only as the “final common pathway” serving as a relay of the final motor program generated by upstream interneuron circuits. In this view, motor neurons function as a readout component that merely conveys motor commands from premotor interneurons to the target muscles. Our recent study in the adult zebrafish invalidated this view and revealed an unforeseen influence of motor neurons via electrical synapses onto premotor excitatory interneurons and the generation of the locomotor rhythm by spinal circuits. By combining whole-cell paired recordings, optogenetics and anatomy in adult zebrafish, we show the existence of bidirectional electrical coupling between motor neuron dendrites and V2a interneuron axon terminals. This endows the motor neurons with the capacity to strongly impact synaptic transmission and firing properties of the premotor excitatory V2a interneurons via backward propagation of electrical signals through gap junctions and as a result influence the performance of the locomotor CPG. These results show that motor neurons are not a passive recipient of motor command but an integral component of the neural circuits for motor behavior.

S6.3

A single motor neuron determines the rhythm of early motor behavior in *Ciona*

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Recent work in tunicate supports the similarity between the motor circuits of vertebrates and basal deuterostome lineages. To understand how the rhythmic activity in motor circuits is acquired during development of protochordate *Ciona*, we investigated the coordination of the motor response by identifying a single pair of oscillatory motor neurons (MN2/A10.64). The MN2 neurons had Ca^{2+} oscillation with an ~80-s interval that was cell autonomous even in a dissociated single cell. The Ca^{2+} oscillation of MN2 coincided with the early tail flick (ETF) observed at stage (St.) 23 to 24. The spikes of the membrane potential in MN2 gradually correlated with the rhythm of ipsilateral muscle contractions in ETFs. The optogenetic experiments indicated that MN2 is a necessary and sufficient component of ETFs. These results indicate that MN2 is indispensable for the early spontaneous rhythmic motor behavior of *Ciona*. Our findings shed light on an understanding of the development and evolution of rhythmic locomotion of chordates.

S6.4

Motoneurons modulate leech motor pattern through central connections

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Motor behaviors are controlled by neuronal networks that are markedly hierarchical and highly distributed. At the top of the hierarchy a few neurons decide global aspects of the behavior, while at its basis, numerous motoneurons control the muscles involved in the behavior. The actual motor pattern is defined by intermediate rhythmogenic networks. In this scheme motoneurons are mere output units. Are they? Leeches are an excellent organism for the study of motor control. On solid surfaces leeches crawl through a sequential series of elongation and contraction body waves, produced by activation of circular and longitudinal muscles, respectively. The motor pattern can be recorded in the isolated nerve cord through the activity of the corresponding motoneurons. Dopamine induces fictive crawling (*crawling*) in the isolated nerve cord and in isolated individual ganglia. This indicates that the network necessary to produce *crawling* is present in each midbody ganglion, including the central pattern generator (CPG). We've shown that motoneurons influence the CPG directly and indirectly: i) the motoneurons that control circular muscles feedback onto the CPG, regulating the phase relationship; ii) a pair of premotor nonspiking (NS) neurons in each ganglion are coupled to all the motoneurons via rectifying electrical junctions and are inhibited by the motoneuron through chemical synapses. This pathway constitutes a recurrent inhibitory circuit (analogous to Renshaw cells circuit in vertebrates) that modulates motoneuron activity. During *crawling* NS membrane potential oscillates in phase with the motor pattern and modulates the motor output. These results show that, in addition to their peripheral targets, motoneurons contribute to shape the motor pattern through central connections.

INVITED SYMPOSIUM 7 – NEW TOOLS TO STUDY BEHAVIOUR IN THE FIELD: INSIGHTS FROM INSECT NAVIGATION

The crux of neuroethology is to consider the neural mechanisms that give rise to complex behaviour in natural environments. Yet, many of the most advanced methods to probe behavioural mechanisms remain consigned to the laboratory. The field of insect navigation is bucking this trend by developing novel methods that blur the lines between laboratory and field studies, delivering significant advances in our understanding. This symposium will bring together world-leaders from technical and biological disciplines to describe new in-field methodologies and the impact they have had on insect navigation research. Talks will offer a perspective on recent trends and look ahead to future directions in order to stimulate debate in the meeting. The focus on emergent technologies and their impact is particularly timely and innovative, with an integrative view ensured by securing speakers from disparate academic specialisms & locations. ICN is the ideal venue for this symposium as its worldwide reach ensures a rare meeting of experts from all corners of the globe with a shared research goal.

S7.1

Quantifying insect behaviour in the wild – Fully automatic tracking and habitat reconstruction from a single hand-held camera

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Ideally, animal behaviour would be studied in its ecological context, but comprehensive fine-scale measurement has required controlled lab conditions. To understand insect navigation, for example, we would like to track individuals throughout their foraging lives, as they take unconstrained and lengthy paths through their natural habitat, with a known level of experience and motivational state. Here we solve the problem of tracking ants from video taken using a hand-held camera (unknown motion) following a very small animal (insufficient pixels to use kernel methods) in natural terrain (uncontrolled background) with no training data (cannot apply machine learning). We reconstruct the entire paths along with the surrounding terrain, allowing us to extract fine-scale features of the behaviour such as speed, stops, and oscillations; and to analyse how they are affected by motivational state, previous experience, and current environment. This opens up many possibilities for new analyses of the navigational behaviour of other species in their natural habitats.

S7.2

The Antarium: Manipulating the visual world of navigating insects

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We constructed a large arena (*the Antarium*) with 20 000 UV-Blue-Green LEDs spectrally matched to ant photoreceptor sensitivities that allows us to present tethered ants with views of their natural foraging environment. The ants are free to rotate on a trackball but their translational movements are registered and can be fed back to the visual panorama. View changes that are due to the ants' translation are generated in a 3D model of the ants' environment (where the movements of Australian bull ants have been recorded with differential GPS), so that they experience the changing visual world in the same way as they do when foraging naturally. In experiment 1, the ants were confronted with four stationary views: along their foraging corridor, at their nest, an unfamiliar scene and a visually unstructured environment. Ants treat each of these views differently and in the case of familiar views change walking direction whenever the panorama is rotated in a step-wise fashion. In experiment 2, ants were confronted with a stationary panorama at their nest, and then their foraging tree, was rotated 90o to the left or to the right of its normal position. Ants in this experiment were unaffected by the displacement of the foraging tree, demonstrating that their heading direction is not determined by individual landmarks, but by the whole panorama. The antarium is a unique instrument to investigate visual navigation in ants, because it allows us to confront ants with familiar and unfamiliar views, with completely featureless visual scenes, or with scenes that are altered in spatial or spectral composition. In future, the antarium can also be used to investigate the dynamics of navigational guidance and the neurophysiological basis of ant navigation in natural visual environments.

S7.3

In field neural manipulations to investigate the basis of working memory for insect navigation

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Today, more than 50 years after the first behavioral evidence that insects use path integration, we still have a limited understanding of how information about direction and distance is integrated in the brain to encode the home vector. To unravel the functional and mechanistic characteristics of the vector memories, we performed a series of experiments on the path integrating dung beetle *Scarabaeus galenus* in its natural environment, in

↑UP

South Africa. We found that, while the directional component of the home vector was maintained for up to one hour, the distance component of the vector memory decreased gradually over time. Neural manipulations performed in the field using cold-induced anesthesia provided us with a unique tool to disrupt the neural activity of beetles that had stored a home vector of known length and direction. This treatment diminished both components of the home vector memory, but by different amounts, overall suggesting that a biological process that can be disrupted by cold-induced anesthesia is essential to support homing by path integration. With that, our study represents a milestone in the growing effort to understand and mitigate the nature and neural basis of insect path integration.

S7.4

Brains-on-board robots: testing embodied neural circuits in the wild

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Increases in computing power in the last decades have led to many advances in the scale and precision with which researchers can model neural signalling. However, an animal's intelligent or adaptive behaviour originates not from the brain in isolation, but emerges from brains actively perceiving and interacting with the environment that they have been evolved in. As such, to accurately assess models of intelligent behaviour, we must test them as they act within the animal's natural habitat. Here, I will review the work of the Brains-On-Board project in which we test how models of insect behaviour deal with real world conditions by implementing neural circuits on-board robots moving through complex natural environments.

INVITED SYMPOSIUM 8 – REDEFINING THE BOUNDARIES OF PHEROMONE ACTION: PHEROMONES AS NEUROMODULATORS OF LEARNING AND MEMORY

The symposium will propose a *redefinition of the concept of pheromone based on novel, cumulative behavioral and neurobiological evidence*. Given the traditional and long-established view that confines pheromones to an exclusive species-wide communication role independent of experience, the discussion about novel pheromonal functions modulating experience-dependent behavior proposed in this symposium constitutes, in our opinion, an innovation in biological thinking. Pheromones are defined as chemical messengers that are released to the environment by a sender and that induce changes in behavior of a receiver of the same species. They constitute the ubiquitous mode of information transfer among animal species and occur in multiple behavioral contexts such as food and mate search, predator avoidance, territoriality and navigation. The response to pheromones is, by definition, stereotyped and independent of experience. Yet, recent work, in both vertebrates and invertebrates, has revealed an unsuspected role of pheromones, namely their capacity to modulate learning and memory formation, beyond the original communication context for which they evolved. Here we propose to focus on this "non-canonical" role of pheromones and discuss if and how pheromones affect cognitive behaviors that are in principle unrelated to the chemical message conveyed. The fact that pheromones may facilitate or inhibit associative learning and memory formation deserves, in our opinion, a broadening of the definition of pheromone action and role, and a discussion of the mechanisms underlying this modulation.

S8.1

Sexual pheromones, reward and learning in female rodents

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In female mice (and rats), testosterone-dependent pheromones released in the urine of males act as unconditioned stimuli that elicit innate behavioural responses. These responses are adaptable depending on the underlying reproductive stage of the females. Pre-pubertal female mice largely avoid male-soiled bedding, whereas aversion shifts to attraction in young adult females, enabling the occurrence of sexual encounters. When a female is lactating, the value of male pheromones shifts again from attraction to aggression, enabling nest defence. Associative learning between male pheromones, detected by the vomeronasal system, and other sensory and spatial cues help the females navigating in a complex social environment. The neural circuits underlying pheromone guided behaviours and learning encompass the projections from olfactory bulbs to the cortical and medial portions of the amygdala, and from those to the ventral striatum, including both the olfactory tubercle and the nucleus accumbens. Manipulation of these circuits leads to significant changes in behaviour. For example, lesions of the anteromedial olfactory tubercle and pharmacological inhibition of glutamatergic transmission in the accumbens significantly decrease the innate attraction of females towards male pheromones. Further, chemogenetic inactivation of the medial amygdala reduces escalated aggression of dams towards male intruders. Finally, it is important to highlight that atypical social behaviour in mouse models of neurological disorders might be due, at least in part, to an aberrant production or processing of chemosignals.
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S8.2

Pheromones modulate learning and memory retention in honeybees according to their valence

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Pheromones have been defined as essential agents of stereotyped communication which release reflexive responses and/or prime physiological effects. However, recent findings have suggested a capacity of pheromones to modulate stereotyped responsiveness to stimuli that are usually considered as reinforcers (reward, punishment), suggesting that they could modulate learning and memory formation. We explored this possibility in the honeybee *Apis mellifera* because its sophisticated social organization and evolutionary success rely largely on pheromonal communication and because of its remarkable learning and memory capabilities. To understand the effect of pheromones, we combined protocols of olfactory conditioning and pheromone pre-exposure with two-photon imaging of olfactory circuits and pharmacological disruption of neural activity in the bee brain. Our results demonstrate that pre-exposure to pheromones affects in a significant way learning and memory, depending on the valence of the pheromone pre-exposed. While pre-exposure to the attractive pheromone improves subsequent olfactory learning and memory, pre-exposure to the deterrent pheromone has the opposite effect. This modulation is not due to changes of olfactory signalling in the bee brain but to changes in appetitive motivation of opposite sign induced by the attractive and the deterrent pheromone. Consistently, interfering with aminergic circuits mediating appetitive motivation in the bee brain rescued or diminished the cognitive effects induced by pheromones. Our results thus suggest that the definition of 'pheromone' should not be restricted to the context of animal communication but should incorporate the capacity of these substances to act as behavioural modulators of motivational and cognitive processes.

S8.3

The alarm pheromone, formic acid, increases nestmate recognition efficiency in ants

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Social insects, ants in particular, are very efficient in discriminating between colony members and aliens. However, no recognition system is immune to error. The acceptance threshold model predicts that rejection (type I) and acceptance (type II) errors are reciprocally related: if one type decreases, the other increases. This prediction has been validated empirically, but we found an important exception. We exposed *Camponotus aethiops* ants to formic acid, an alarm pheromone, and subsequently measured aggression toward nestmates and non-nestmates. Formic acid induced an increase in non-nestmate rejection as well as in nestmate acceptance, compared to a control treatment. Therefore, Nestmate discrimination accuracy was improved via a decrease in both types of errors, a result that cannot be explained by a shift in the acceptance threshold. Furthermore, we found that formic acid decreased the latency of the aggressive response to non-nestmate odours, meaning that ants exposed to formic acid became faster in aggressing intruders. Contrary to the prediction of the speed–accuracy trade-off theory, ants are both more accurate and faster under the effect of formic acid. These results reveal an unexpected and novel function of alarm pheromones in recognition processes.

S8.4

Circuits and mechanisms of pheromone-evoked courtship behavior in the mouse

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Courtship behavior requires functioning of a variety of neural computations including sensation, interoception, learning, and memory. How and where these functions alter behavior is largely unknown. The Stowers lab is leveraging the ability of specialized olfactory cues, pheromones, to specifically activate and therefore identify and study courtship circuits in the mouse. We are interested in identifying general circuit principles (specific brain nodes and information flow) that are common to all individuals, and to additionally study how experience, gender, age, and internal state modulate and personalize behavior. We are solving the structure and logic of two complete sensory to motor courtship circuits to compare and contrast their strategies of how the brain generates innate behavior.

PLENARY SESSION 5

Rhythms in a songbird brain: biomechanics and neural dynamics

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Birdsong is a complex motor activity that emerges from the interaction between the central and peripheral nervous systems, with the body and the environment. The similarities with human speech, both in production and learning, have positioned songbirds as highly useful animal models to study this learned motor skill. In this talk I will show an interdisciplinary approach to study the emergence of behavior. Specifically I will discuss a biomechanical model for song production and its validation through neural experiments. I will show how this model can be used to make predictions regarding motor control and how it can suggest a hierarchy of importance of the physiological parameters. I will also show neuronal recordings in a telencephalic region where a sensori-motor integration occurs, showing the presence of well-defined oscillations in local field potentials, which are synchronized to the rhythm of the canary (Serinus canaria) song. I will also show that there is a correspondence between local field potentials, multiunit activity, and single unit activity within the same brain region. A low-dimensional mathematical model for a neural network will be presented that can reproduce the neural dynamics observed in the experiments. This interdisciplinary work shows how low-dimensional models can be a valuable tool to study the neuroscience of perception, generation and control of complex motor tasks.

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THURSDAY, 28 JULY 2022

PLENARY SESSION 6

Exploring the neural geography of the social brain using medaka fish

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Some fish species have the ability of individual recognition and individuals appropriately tailor attitudes and responses to other group members according to the social context. The neural substrate that works between sensory input and behavioural output underlying social cognition and decision-making processes, however, is vast and mysterious. To address this issue, we have focused on medaka fish, a model animal used mainly in the field of molecular genetics. Previously, we demonstrated that medaka females recognize familiar males following prior visual exposure, and social familiarity influences female mating receptivity. Medaka females exhibit a positive response (high receptivity) to familiar males and negative response (low receptivity) to unfamiliar males. In this talk, I would like to introduce how we determined the neural substrate which could modulate behavioural-choice processes using molecular genetics.

INVITED SYMPOSIUM 9 – MAKING BIOROBOTS BEHAVE: CONNECTING ENGINEERING AND ANIMAL BEHAVIOR

In the last decade there have been important advances in our understanding of animal movements that have helped engineers to design more capable and adaptive robots. To build these bio-inspired machines, engineers are exploring new scientific and technological approaches that are not widely used in biology. This includes the emerging fields of soft robotics, evolutionary robotics and computational simulation. This symposium will highlight the advances being made in bio-robotics and the challenges of building machines that behave like animals. Speakers will focus on the impact of neuromechanics and embodiment on the design and control of robots. These engineering approaches have in turn yielded important insights and tools that can be applied to neuroethological problems. A major goal of the symposium is to bring together engineers and biologists working on the mechanisms of adaptive behavior to discuss the most recent cutting-edge research in their respective fields. Understanding how animals navigate and move around in the world is now having a major impact in the field of robotics. We will present the leading edge of research into bioinspired sensors, soft materials and neural control systems and their application to robots designed to operate in natural environments. Key questions will include the role of central commands and distributed controls in complex movements, biomechanical interactions between animals and their environment and how evolutionary processes can shape an animal's body and behavior.

S9.1

Active touch sensing in mammals and robots

Tony Prescott[1]; Dr. Martin Pearson[2]; Dr. Robyn Grant[3]; Dr. Ben Mitchinson[4]; Dr. Uriel Martinez-Hernandez[5]; Prof. Nathan Lepora[6]
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Active sensing adjusts the movement of the sensor to maximize information gain in relation to the current task. Research on active sensing is demonstrating the complementarity of analytic approaches in the biological sciences and synthetic approaches in engineering, with robots able to physically instantiate and test theories of active sensing developed through this interaction. To better understand active sensing we need to think of sensing and movement as coordinated activities that are part of the closed-loop interaction of the organism with its world. Studies of animals and robots suggest common strategies of evidence accumulation towards a decision threshold, low resolution scanning to identify potential targets for attention, orienting and high-resolution foveation for inspection, and control of the sensing device to keep signals within a sensitivity range. Robots, like animals, benefit from the use of learning to acquire effective control policies, adjust priors (expectations), and to predict the sensory consequences of movement. This talk will review research on vibrissal touch sensing in a range of mammals, including rodents and marsupials, and will explore how the development of robots with biomimetic whisker sensory systems can cast light on some of the principles and processes underlying biological active touch. Active perception will be analyzed using the framework of Bayesian sequential analysis, and investigated via computational neuroscience models of the layered control architecture of the brain. The talk will be illustrated with video recordings of mammalian active sensing and of whiskered robots using active touch for texture/shape recognition and for tactile spatial navigation.

S9.2

Slow motion in the air: aerodynamic load control and sensing in insect wings

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Flying animals can slow down gracefully in the air and come to a dead stop upon landing, in stark contrast to airplanes which perform most of the deceleration after touch-down. This aerial slow motion is enabled by careful control of forces generated from each wing (aerodynamic loading). While many animals transition to a hover before landing, some exploit their compliant wings through active and passive wing deformation to parachute down, minimizing the energetic cost of this behaviour. This talk uses parachuting as a behaviour context to explore the biomechanics of highly deformable wings and the implicated functions of wing mechanosensory systems. Most insect wings are highly deformable (i.e. soft), has many passive degrees of freedom (i.e. under actuated), and yet populated with numerous sensors placed strategically. Controlling such soft membrane with only a few active degrees of freedom at the wingbase examplifies a typical challenge in soft robotics. Thus, insect wings are an excellent model for studying biological control of soft structures and the role of aerodynamic load sensing. Firstly, I will demonstrate how the changes of wing configuration and joint compliance in the dragonflies enable them to perform aerial righting during vertical descent with minimal effort. Secondly, I will review a complete map of wing sensory system in the dragonfly and highlight an on-going modelling work on wing load sensing. Finally, I will present an approach to parametrize complex wing aeroelastic deformations and how it allows the interpretation of neural signals from some wing mechanosensors. As my talk journeys through the parachuting behaviour, wing biomechanics, wing sensor distribution, and wing sensory encoding, I hope to illustrate how biomechanics of compliant stuctures can shape the implementaiton of behaviours at a fundamental level.

S9.3

Exploring the interaction of feedforward and feedback control in the spinal cord using biorobots

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The ability to efficiently move in complex environments is a fundamental property both for animals and for robots, and the problem of locomotion and movement control is an area in which neuroscience, biomechanics, and robotics can fruitfully interact. In this talk, I will present how biorobots and numerical models can be used to explore the interplay of the four main components underlying animal locomotion, namely central pattern generators (CPGs), reflexes, descending modulation, and the musculoskeletal system. Going from lamprey to human locomotion, I will present a series of models that tend to show that the respective roles of these components have changed during evolution with a dominant role of CPGs in lamprey and salamander locomotion, and a more important role for sensory feedback and descending modulation in human locomotion.

Furthermore, the models suggest that there is an interesting redundancy between sensory feedback loops and CPGs that provide strong robustness against neural lesions. If time allows, I will also present a project showing how robotics can provide scientific tools for paleontology.

S9.4

Navigation in insects and robots

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Insects have long been a source of inspiration for flying robot control, and offer the possibility of reliable, lightweight autonomous navigation solutions. Insects such as bees are known to be able to navigate between sites of interest in large local neighbourhoods that span several kilometres, using relatively low-resolution visual input and computational processing performed in a relatively small brain. We describe several hardware and software developments resulting in an aerial biorobot that we use to test insect navigation models, featuring a quadcopter airframe, Pixhawk flight controller and an active mechanical view stabilisation system. This biorobot is first used to embody a recently proposed anatomically constrained path integration circuit. It uses a biologically plausible matched visual filter to obtain ground speed during holonomic flight, and combines this with directional information in the neural circuit to maintain an estimate of its starting position. We show this real-world system, tested outdoors, has a homing error drift rate of only 1.5m per 100m flown. A new design for a polarisation-sensing system, based on the receptor layout of the insect eye, has also been implemented and can be used to obtain an accurate 360 degree estimate of heading. We have also used the biorobot to investigate whether flying insects could use visual route following to overcome the drift issues associated with path integration. We propose a new approach using downward views and a similarity algorithm based on oriented bandpass filters that plausibly represent processing in the insect visual system. We show this method can be effective to zero in on the target location across distances of at least 30m, even in seemingly featureless environments such as empty arable fields.

INVITED SYMPOSIUM 10 – INSIGHTS INTO THE FINE TUNING OF SOCIAL BEHAVIOR: THE BRAIN AS A SOURCE OF STEROID HORMONES

The aim of the symposium is to bring together data from four research lines on steroid modulation of sexual and aggressive behavior carried out in mammals, birds and teleost fish, to illustrate how studying behavior and the social brain areas involved across sexes and seasons has brought forth new ideas on hormone modulation of behavior. Hormones, key agents of biological coordination, have long been known to affect and be affected by behavior. In the last twenty years, novel data have emerged that contribute to the existing foundation built upon the study of the role of steroid hormones in male breeding behavior. Current approaches have included three non-traditional standpoints: 1. female aggression 2. social behaviors uncoupled from the breeding season and 3. the effects of brain-derived hormones on aggressive and sexual behaviors. Research with these focuses has opened new avenues to understand the diversity of steroid modulation upon social behavior.

S10.1

Neurosteroids and territorial aggression in a songbird

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Steroids profoundly influence many behaviours, including feeding, aggression, migration, reproduction, and parental behaviour. Neuroethologists and neuroendocrinologists have traditionally focused on circulating steroid levels in the blood. However, circulating steroid hormones are converted to more active and less active signaling molecules within specific brain regions. Furthermore, brain regions can synthesize steroids from cholesterol or circulating precursors. Therefore, steroid levels in the blood and specific brain regions can differ dramatically. In birds, neural steroid synthesis regulates neurophysiology, structural neuroplasticity, vocal communication, and territorial behavior. In particular, male song sparrows (Melospiza melodia) demonstrate robust territorial aggression during the breeding season and non-breeding season, although the testes are regressed and circulating testosterone levels are non-detectable during the non-breeding season. During the non-breeding season, a simulated territorial intrusion rapidly increases testosterone levels in specific brain regions, and steroid treatment rapidly increases aggressive behaviour. These data suggest that neurosteroids modulate territoriality, an important and complex social behaviour.

S10.2

Winter madness: The neuroendocrine regulation of seasonal aggression

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Individuals of virtually all vertebrate species are exposed to annual fluctuations in the deterioration and renewal of their environments. As such, organisms have evolved to restrict energetically expensive activities to a specific time of the year. The precise timing of physiology and behavior is critical for individual reproductive success and subsequent fitness. Although the majority of research on seasonality has focused on reproduction, pronounced fluctuations in other social behaviors, including aggression, also occur. To date, most studies that have investigated the neuroendocrine mechanisms underlying seasonal aggression have focused on the role of photoperiod; prior findings have demonstrated that some seasonally breeding species housed in short "winter-like" photoperiods display increased aggression compared with those housed in long "summer-like" photoperiods, despite inhibited reproduction and low gonadal steroid levels. While fewer studies have examined how the hormonal correlates of environmental cues regulate seasonal aggression, our research suggests that the pineal hormone melatonin acts to increase non-breeding aggression in Siberian hamsters (*Phodopus sungorus*) by altering steroid secretion. This talk addresses the physiological and cellular mechanisms underlying seasonal plasticity in aggressive behavior, and proposes a key role for melatonin in facilitating a "seasonal switch" to alternative physiological mechanisms of aggression across the annual cycle. Collectively, these findings will highlight novel and important mechanisms by which melatonin regulates aggressive behavior in vertebrates and provide a more comprehensive understanding of the neuroendocrine bases of seasonal social behaviors more broadly.

S10.3

Role of neuroestrogens in the control of male sexual behavior

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The conversion of testosterone by brain aromatase is necessay for the activation of several physiological responses and behaviors, including male sexual behavior. Brain-derived estrogens (neuroestrogens) are typically thought to participate to the long-term regulation of behavior by seasonal changes in circulating testosterone, while the moment-to-moment regulation of behavior in response to environmental/social cues would depend on neurotransmitter systems. This view fits in with the classical mode of action of sex steroids which regulate gene expression by acting on nuclear receptors resulting in behavior changes observed within hours to days. Yet, estrogens also exert much faster actions mediated by the activation of membrane-associated receptors that rapidly modulate cellular activity translating behavioral changes detected within minutes. In parallel, brain estrogen production is also rapidly regulated at the presynaptic level by behaviorally relevant cues, thus providing a mechanism for fast and local regulation of estrogenic actions. This talk will summarize our work carried out in Japanese quail demonstrating the dichotomy between these two modes of action of neuroestrogens and how they cooperate to the fine coordination of male sexual behavior within two distinct time frames. Finally, more recent work conducted in mice will also be presented.

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S10.4

Neuroendocrine modulation of aggression: contributions from a wild electric fish

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In all vertebrate classes, agonistic behavior is an adaptive social behavior that plays an important role in gaining access to limited resources. The physiological mechanisms underlying aggression have mostly been examined in breeding males, in which gonadal androgens, acting in part through their aromatization to estrogens, have a key role. Nevertheless, aggression may occur uncoupled from reproduction, suggesting a role for alternative non-gonadal regulatory mechanisms, particularly those involving brain-synthesized hormones. This talk will address the contribution of a traditional neuroethological model, the weakly electric banded knifefish, to understanding the neuroendocrinology of territorial aggression. *Gymnotus omarorum* displays year-round territorial aggression in both males and females. Non-breeding aggression is independent from gonadal hormones, however, estrogens have a prominent role in its regulation. Quantification of plasmatic and forebrain steroid hormones shows that estrogens are exclusively brain-derived in the non-breeding season in both males and females. Moreover, there is gene expression of aromatase, as well as estrogen and androgen receptors, during the non-breeding season in key areas of the social brain network. Overall, the data point to neuroestrogens as important modulators of non-breeding aggression acting in regions of the social brain through rapid mechanisms, a strategy that has been shown to be common across distant vertebrate species.

INVITED SYMPOSIUM 11 – NEUROETHOLOGY OF 3D SPATIAL NAVIGATION

This symposium aims to present and discuss the role of hippocampus in representing 3D space during navigation across a diverse set of mammalian taxa – rodents, lemurs, bats and primates. Specifically, the speakers will highlight both the similarities and differences in hippocampal function evident across these organisms and their respective relationship to the species' ethology. The presented data will also combine methodologies spanning from ethological studies in the animal's natural environment, computational modeling and experimental neurophysiological interrogation. Combined, this symposium will highlight the importance of a comparative approach in studies of the neural mechanisms underlying spatial navigation.

S11.1

Closed-loop neuroethology in freely foraging mouse lemurs

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I will present wireless recordings from the hippocampus in mouse lemurs freely foraging in three-dimensional naturalistic environments. I will also discuss the EthoLoop system, a novel framework able to track movements and analyze behaviors, while providing stimuli and rewards in a closed-loop manner to reinforce specific behaviors.

S11.2

Environmental influences on the neural encoding of 3D space – insights from rats

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How the mammalian brain makes an internal (cognitive) map of space has been intensively investigated since the seminal discovery of place cells 50 years ago. Studies in flat, 2D (two dimensional) environments have revealed the existence of (as well as place cells) head direction cells and grid cells, which supply direction and distance information respectively. It was initially assumed that the same encoding properties would apply in naturalistic 3D spaces as apply in 2D. This talk will outline a set of experiments in rats that show that the properties in 3D are different from those in 2D, and also that environment structure greatly shapes the nature of the cognitive map. The structure of the map is thus not fixed, but is flexibly constructed as a result of interactions between the animal and its environment.

S11.3

Representing space in marmoset hippocampus

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Vision and audition are the dominant sensory modalities for primates and play critical roles in how they explore their environments. Here we will report on a series of experiments in marmoset monkeys aimed at understanding how ego- and allocentric representations of space are constructed from both visual and auditory information in the primate hippocampus. Our experiments on vision take advantage of a novel head-mounted eye-tracking system developed in my lab that allows us to precisely measure the eye movements of freely-moving marmosets, while recording hippocampal activity. Experiments focused on auditory space complement this naturalistic approach by examining the activity of hippocampal neurons while freely-moving monkeys engage in natural conversational exchanges in a dynamic, cocktail party environment. These complementary lines of work seek to better understand how primates construct representations of space as active agents navigating the world that receive sensory and spatial information from multiple modalities.

S11.4

Neural representations across time and space in the hippocampus of freely flying bats

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Our lab seeks to understand the neural basis of complex spatial behaviors in mammals. To do so, we take a neuroethological approach that leverages the specialization of the bat (*Rousettus aegyptiacus*) for 3D spatial movement and in particular, its ability to elegantly navigate at high spatial precision during high-speed flight and across different sensory conditions. In parallel, we pioneer a suite of cutting-edge technologies that make it possible to study the behavior and neural circuits in freely flying bats in ways not previously possible. In this talk, I will focus on the neural representation in the hippocampus of flying across space and time. I will discuss findings addressing how the hippocampus represents spatial information during aerial navigation on both short (milliseconds/seconds) and long (days/weeks) timescales.

INVITED SYMPOSIUM 12 – SELECTIVE ATTENTION AND STATE-DEPENDENCY IN INVERTEBRATES

The classical model of sensory behaviour posits that an organism receives an external stimulus which then elicits a specific response in a stereotypical fashion. However, several recent studies have shown that an organism's response is not stereotypical but also depends on its behavioural state. Such state-dependent responses have been shown in a range of species including primates, mice and insects. Importantly, these state-dependent responses have been argued to be analogous to responses that are dependent on attention. Thus, behavioural states, such as flying or walking, could be on a continuum with more psychological states such as attention. The study of selective attention in invertebrates has, however, progressed relatively independent of the study of state-dependent behaviour. Much recent progress in the neural and genetic basis of selective attention in multiple systems shows that this is an important, growing field. The goal of this symposium is to bring together

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experts in these two fields to discuss how state-dependency and selective attention could inform each other and how we can enhance studies in both fields by sharing theoretical ideas and techniques.

S12.1

Short-term water deprivation modulates hygrosensory and visually-evoked behaviors in flying flies

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Organisms must integrate ever-changing external environments with internal physiological states to generate behaviors that support survival. We aim to understand strategies that permit dynamic representations of sensory information that underlie flexible behavioral outputs in varied internal states. For example, dehydration is a persistent threat to the fruit fly, *Drosophila melanogaster* due to their large surface area to volume ratio. Do flying *Drosophila* alter the value of water to seek it out in a dehydrated state? We utilize a virtual reality flight simulator that permits flying flies to rotate freely in response to controlled sensory stimuli. While well-hydrated flies assign a neutral value to a water plume in flight, we find that acutely dehydrated (3 hrs) flies alter the salience of and assign a positive value to water cues in order to generate water-seeking behavior. In addition to modifying hygrosensory behavior, we also find hydration-state dependent changes in visually-evoked behaviors in flight. These findings provide a foundation for further examination of how neural circuits in the brain integrate internal physiological state to modify perception across sensory modalities to reliably generate contextually appropriate behavior.

S12.2

Muscles that move the retina augment compound-eye vision in *Drosophila*

Lisa Fenk[1]; Sofia Avritzer[2]; Jazz Weisman[2]; Aditya Nair[3]; Lucas Randt[1]; Thomas Mohren[2]; Igor Siwanowicz[4]; Gaby Maimon[2]

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The majority of animals have compound eyes, with tens to thousands of lenses attached rigidly to the exoskeleton. A natural assumption is that all these species must resort to moving either their head or body to actively change their visual input. However, classic anatomy has revealed that flies have muscles poised to move their retinas under the stable lenses of each compound eye. We found that *Drosophila* use their retinal muscles to both smoothly track visual motion, which helps to stabilize the retinal image, and also to perform small saccades when viewing a stationary scene. We show that when the retina moves, neuronal receptive fields shift accordingly and that even the smallest retinal saccades activate visual neurons. Using a new head-fixed behavioral paradigm we find that *Drosophila* perform binocular, convergent movements of their retinas—which could enhance depth perception—when crossing gaps and impairing retinal-motor-neuron physiology alters gap-crossing trajectories during free behavior. That flies evolved an ability to actuate their retinas argues that moving the eye independently of the head is broadly paramount for animals. Active retinal movements provide a previously unappreciated way for improving insect vision across space and time.

S12.3

A role for active sleep in regulating selective attention and evolving consciousness

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Like most other animals, fruit flies sleep and their sleep seems to be partitioned into distinct stages. Using electrophysiology as well as calcium imaging in *Drosophila*, we have uncovered an 'active' sleep stage alternating with a 'quiet' sleep stage in the fly brain, which we propose are evolutionary antecedents of REM and slow wave sleep, respectively. These sleep stages can also be induced pharmacologically or by activating specific neurons in the fly brain. During active sleep the fly brain seems awake except that animals are rendered mostly unresponsive to external stimuli. During quiet sleep neurons in the fly brain become less active while glia are more active, and regular probiscis extensions perform a waste clearing role. RNA sequencing suggests that an entirely different set of functions are being engaged by active sleep, compared to quiet sleep. In humans REM sleep has been associated with emotional regulation. How to test whether a similar link might exist in flies is not obvious. Understanding that emotional regulation in humans is centred on optimizing responses to surprising events or prediction errors, we developed a simple visual paradigm where stimuli had different levels of predictability, and investigated responses in the fly brain during spontaneous sleep as well as during induced active sleep. We also examined behavioural responses after flies had experienced an extended bout of active sleep, compared to flies that had experienced mostly quiet sleep or spontaneous sleep. We found that active sleep corrected visual attention defects incurred by sleep deprivation. This suggests a conserved role for active sleep in regulating selective attention, which we propose was key to the evolution of consciousness in animals.

S12.4

Biophysics of mechanosensory perception is tuned both by internal behavioural states and external environmental states in crickets and spiders

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One view of perception is that information travels from the environment to the sensory system, to the nervous systems which processes it to generate a percept and then behaviour. Ongoing behaviour is thought to occur largely through simple iterations of this process. In this talk I will show that at least in some sensory modalities, such a view is untenable given the underlying physics. I will present data which shows that in some systems the physics of the sensory system dictates that perception will be altered. In the case of tree cricket, auditory perception is inevitably affected by the environmental temperature, and in spider's vibration perception is inevitably affected by locomotor behaviour. In crickets, ambient temperature alters changes the properties of the molecular machinery that underlies hearing, thus altering the tuning properties of auditory neurons. In spiders I will present data on the physical configuration of body parts determines the transmission and delivery of vibrations to the sensory organs. I hope these data will show that state dependency may not always be an optional feature but may be an inevitable consequence of the fact that perceptual systems are built from physical objects with inherent physical properties.

PLENARY SESSION 7

Visual world of flower foraging swallowtail butterflies

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Flower foraging swallowtail butterfly *Papilio xuthus* strongly relies on its vision to detect flowers. *Papilio* butterflies trained to take nectar at a target of a certain color can accurately discriminate differently colored targets solely based on their chromatic contents. Even in the light environment changing from time to time, *Papilio* can discriminate colored targets, indicating that they have color constancy. Moreover, the perception of color and brightness of the targets depends on the background's color and lightness, respectively; i.e., *Papilio* has simultaneous color contrast and brightness contrast. In addition to these basic color vision abilities, foraging *Papilio* can discriminate e-vector angles of polarized light as brightness differences. This polarization vision presumably enhances the brightness perception, which compensates for the low spatial resolution of the compound eye system. Mechanistically, *Papilio*'s color vision is tetrachromatic based on the ultraviolet, blue, green, and red-sensitive photoreceptors in the compound eye. The performance of the tetrachromatic system is outstanding: *Papilio* can discriminate only one-nanometer difference at three regions in the wavelength range from ultraviolet to red, which outperforms humans. The high acuity suggests the existence of multi-spectral neurons in higher brain regions. In *Papilio*, the mushroom body, known as the center for learning and memory in the insect brain, receives visual inputs containing a wide variety of spectrally opponent and sharply-tuned neurons. These neurons resemble the color-encoding cortical neurons in primates, suggesting that the mushroom body is responsible for the acute wavelength discrimination and learning abilities when visiting flowers.

WALTER HEILIGENBERG LECTURE

The biased brain: How the owl knows what to rely on for sensory perception

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Many owls are nocturnal birds of prey with specialized sound localizing behavior and adapted brain circuits. Barn owls as model organism have provided insights into mechanistic and evolutionary brain properties at high levels of clarity, inspiring hypotheses that have been tested across species. Particularly, the optimality of brain function with regards to natural behavior is a fundamental property driving adaptation and survival of species. For example, while owls accurately localize sounds near the center of gaze, they systematically underestimate the azimuth of sound sources in the periphery. Other species including humans also underestimate peripheral sound directions, and similar perceptual biases can be found across modalities. Many of these biases may be beneficial for the fitness of a species. Owls offer an advantageous system for testing whether and how biases reflect optimal encoding of natural statistics about environment and sensory inputs in the brain, including the integration into behavioral-command neural signals and adaptation by experience. Hypotheses from this research are also being tested in humans, underscoring the importance of predicting natural statistics to optimize brain function for the benefit of a species' fitness.

FRIDAY, 29 JULY 2022

PLENARY SESSION 8

Cellular, molecular, and physiological adaptations of hibernation

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Mammalian hibernation is fascinating. During a short period of time, hibernating animals undergo dramatic adaptive changes, including a reduction in heart and respiration rate and a decrease in core body temperature from 37°C (98.6°F) to 4°C (39°F), yet they do not experience cold-induced pain, and their organs continue to function despite being cold and deprived of oxygen for 8 month out of the year! Moreover, since these animals do not eat or drink during hibernation, they must rely solely on the management and utilization of their internal resources for long-term survival. How hibernators achieve such a remarkable physiological adaptation, remains unknown. We use hibernating 13-lined Ground squirrels (an obligatory hibernator) and Syrian hamsters (a non-obligatory hibernator), to tackle fundamental biological questions from perspectives unachievable using the standard animal models alone. Specifically, we are interested in studying molecular evolution of mammalian hibernation and cellular adaptations that these animals evolve in order to survive prolonged periods of hypothermia, water deprivation and starvation. We are also trying to pinpoint the molecular and physiological basis of hibernation induction. Comparative analysis of three rodent species—such as ground squirrels, hamsters and mice (non-hibernator)—at the behavioral, cellular and molecular levels, will help us to delineate the multitude of adaptations that hibernators evolved in order to survive harsh environment and as a result came to inhabit a wide geographical range.

PARTICIPANT SYMPOSIUM 5 – MOTOR CONTROL II

T5.1

Visual and antennal mechanosensory feedback affect head stabilization in hawkmoths

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Across animals, gaze stabilization helps in reduction of motion blur during rapid maneuvers. Insects use compensatory head movements for stabilizing gaze because their eyes are fixed to their heads. The sensory mechanisms mediating head stabilization are well elucidated in flies where the feedback is primarily derived from vision and halteres, the hindwing equivalent in flies. What mechanosensory structures mediate gaze stabilization in non-Dipteran insects? The question is more pertinent in nocturnal insects because they perform gaze stabilization under low light conditions. Previous studies have indicated that antennal mechanosensors may serve to provide mechanosensory feedback that is crucial for flight stability in moths and also, play an important role in mediating head roll in walking crickets. In a series of experiments in tethered moths, we examined the role of antennal mechanosensors and visual feedback in maintaining head stability of the nocturnal hawkmoth, *Daphnis nerii*. Our experiments in light and dark conditions at varying frequencies of imposed roll revealed that visual cues are primarily important at low frequency. Antennal manipulation experiments further showed that antennal mechanosensory feedback strongly affects head stabilization, and is mediated by *Johnston's organ* located at the antennal base. Additionally, we observed small-amplitude head wobble movements, which also existed in stationary flying insects. The head wobble was affected by sensory feedback. Together, these experiments illustrate that the integration of visual and antennal mechanosensory feedback help the moths in maintaining a stable head position during flight.

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T5.2

Coordination and causal mechanisms for neural control of flight in a comprehensive hawkmoth motor program

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Flying insects face a daunting control problem where as few as 10 muscles must be activated by the nervous system in a temporally precise and coordinated manner to stay airborne and perform maneuvers. It is challenging to unravel how the nervous system coordinates precise spike timing changes across these muscles to alter flight. Are there consistent patterns of coordination that arise for specific maneuvers, and can features of these be reproduced by changing the timing of only specific muscles? To answer these questions we recorded spike-resolved EMGs from 10 muscles representing a comprehensive motor program for flight in the hawkmoth *Manduca sexta*. With a combination of linear decoding of coordination patterns and causal manipulation of spike timing through stimulation, we sought to discover likely coordination patterns present for generating turning maneuvers and investigate if these correlational patterns could be causally reproduced. We found that through a linear decoding pipeline, spike covariation present in one kind of turning maneuver can be used to decode behavior in totally different maneuvers, indicating conserved coordination patterns at the level of motoneuron timings. We chose one particular pattern observed, a change in main downstroke muscle timing associated with pitch turns, and causally induced changes in this muscle timing to causally verify the coordination pattern and study whether individual power muscle timing can lead to maneuvers. We observed a causally-induced association between downstroke muscle timing and pitch torque, but with large unexplained variation indicating the importance of coordination across the motor program for turning maneuvers.

T5.3

Electrophysiological recordings in a running crab show the role of a lobula giant neuron in the speed control of the escape response to visual stimuli

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The crab *Neohelice* is an established model for studying the neural basis of escape behaviors. Four different classes of lobula giant neurons are thought to conform a chief microcircuit involved in the visual control of the crab´s escape. One of these classes named MLG2, that is likely represented by a single element per lobula, proved to be capable of performing the visuomotor transformation involved in adjusting the running speed of the crab to the dynamic of looming stimuli. The results were obtained by matching the temporal course of behavioral responses recorded in a treadmill device to looming stimuli and intracellularly recorded responses to those stimuli obtained in separate immobile individuals. Recently we succeeded in making tetrode recordings in animals performing on the treadmill. Here we show results of simultaneously recorded escapes and MLG2 responses. First, our results confirm that the dynamic of looming expansion is reflected by the firing rate of the MLG2 and matches the speed of escape run. Second, we found that the initial response of the MLG2 to looming stimuli allows to predict the final escape velocity. Third, animals that escaped at greater speeds have MLG2 neurons with higher spontaneous firing rates . Fourth, after the end of stimulus expansion the MLG2 continued firing in proportion to the running speed when the animal was allowed to escape but suppressed its firing when the animal was prevented to escape. This reveals that the response of MLG2 has a motor activity dependent modulation. Altogether, these results provide strong new evidence on the key role of the MLG2 in the regulation of the crab´s running speed to visual stimuli.

T5.4

A population of descending neurons mediating the optomotor response in flying *Drosophila*

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Flying animals must execute continuous trimming adjustments to compensate for internal and external perturbations, such as morphological asymmetries of the body or sudden changes in directional airflow. Prior work in *Drosophila melanogaster* identified a nearly homomorphic population of ~15 descending neurons, the DNg02s, that collectively regulate wing stroke amplitude over a large dynamic range and may be involved in visually-mediated flight stabilization. We present additional findings illuminating the precise function of these neurons in flight control. Our experiments exploit the optomotor response, a behavior in which fruit flies steer to minimize wide-field optic flow, which can be quantified in tethered flies as a difference in the left and right wing beat amplitudes. Using 13 split-GAL4 driver lines to selectively silence various subsets of the population, we find a clear negative linear relationship between the number of DNg02 neurons silenced and the measured optomotor response, suggesting the use of population coding. We then perform two-photon functional imaging on five of the DNg02 driver lines during flight, while presenting optomotor stimuli simulating rotational and linear motion. Depending on the stimulus, the cells exhibit both bilaterally symmetric and asymmetric patterns of activity, and both increases and decreases in activity. Current work aims to untangle the roles of individual cells within the population, identify any subpopulations present, and derive the upstream and downstream connectivity from visual inputs to the system to motor outputs.

T5.5

A model of harvester ant grasping behavior

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Ants use tactile sensing to feel items of food, and after a short period of time, will attempt to grasp the food using their mandibles. If the ant fails, it will make repeated attempts until it has a stable hold on the object. Thus, ants demonstrate successful manipulation of unknown, arbitrarily shaped objects with very little sensory information, a feat still difficult for robots. The key subject of this work is how different motion control methods used by ants' antennae relate to the quality of tactile data gathered, and how these data relate to chance of grasp success when manipulating objects of unknown shape. Therefore, we present a MATLAB-based system that provides a customisable structure for the simplified kinematic, motion control, and sensing models of a harvester ant. To represent ant grasping, this model allows the use of different quality measures to initiate a grasp based on the gathered tactile data from the antennae, and later, from the mandibles. The result is a platform that can be modified to include different motion, sensing and grasp evaluation blocks for comparative purposes. Future analysis will evaluate the average grasp quality that emerges from various combinations of antennal motion control, tactile data evaluation, and grasp selection methods. By making a standardized modelling platform that simplifies the kinematic model of the ant, it may be possible to have more generalized descriptors of ant antennal sensing behaviour. These generalized descriptors will be crucial in identifying the features of ant behaviour that lead to their grasping success.

T5.6

Rapid color change for camouflage in two benthic predatory fishes

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For a successful hunt, marine ambush predators such as the scorpionfish need to be well camouflaged in the eyes of their prey. This can be achieved by background matching, where fish luminance, color and pattern are similar to the background. To match a variety of backgrounds in a heterogenous environment, some fish can even dynamically change color for camouflage. This change can happen rapidly, over seconds to a few minutes, or slowly, over several minutes to hours or even days. We tested whether two Mediterranean scorpionfish species of the genus *Scorpaena* can rapidly change luminance and color. Fish were alternately placed on backgrounds of different luminance and color and changes over five minutes were documented using calibrated image analysis. We analyzed images from the visual perspective of two potential prey fish, a di- and a trichromat. Both scorpionfish species were able to adapt body luminance and color to the different backgrounds in less than a minute, but contrast against the background stayed above detection threshold for the two modeled observers, indicating a poor background match. Changes in luminance were stronger than changes in color. Visual modeling showed color contrast to the background above detection threshold for the trichromatic observer, but contrast below detection threshold for the dichromatic observer. This highlights the importance of modeling observers with different visual systems to relate camouflage strategies like color change to ecological interactions. Further observations show that scorpionfish change also body pattern in response to the background. Future studies will show how flexible this pattern adjustment is and whether scorpionfish crypsis involves not only background matching, but also disruptive coloration.

T5.7

Predictive saccades for decision making in saffron robber fly (*Laphria saffrana*), a beetle predator

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Predictive gaze control is used by humans, bats, archerfish and salamanders to compensate for intrinsic sensorimotor delays, and secure contact with a fast-moving target. Unlike internal predictions about the sensory consequences of self-motion, which can be easily accounted for with corollary discharges, predicting the future location of an external (independent) target requires an internal model. In this work we show that the robber fly *Laphria Saffrana* uses both relative angular position and velocity to predict the future location of their target and inform the subsequent head saccade when target tracking. This finding may seem surprising, because: (i) robber fly photoreceptors are among the fastest so far studied, (ii) their behavioral latencies are short (≈ 18 ms), and (iii) a reactive feedback systems is sufficient to explain the predatory trajectories of other flies (e.g. the robber fly *Holcocephala fusca*). Here, we show how the predictive gaze control of *Laphria saffrana* for the tracking of moving targets supports the difficult categorization and perceptual decision task of differentiating a beetle from other flying insects, with a low spatial resolution retina.

PARTICIPANT SYMPOSIUM 6 – ELECTROSENSORY SYSTEM, AUDITORY SYSTEM AND VOCAL COMMUNICATION

T6.1

Deviance detection in auditory brainstem responses of bats

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The capability to detect unexpected acoustic cues in an environment of repetitive sounds is of major importance for animals. The neural correlate of this ability is called deviance detection and describes a change in neural response strength that is solely caused by the stimulus' probability of occurrence. Former studies could demonstrate the presence of deviance detection in both animals and humans across different areas of the brain. However, most of these studies focussed on higher-order stations of the auditory system, like the cortex and thalamus, while less is known about the role lower stations play in the processing of acoustic probability-encoding. With the current study, we aimed to tackle this issue by measuring deviance detection in auditory brainstem responses (ABRs) of the bat species *Carollia perspicillata*, a hearing specialist. Our results demonstrate significantly increased responses to unexpected deviant compared to expected standard stimuli already at the level of the brainstem, an auditory station that was previously not known to exhibit deviance detection. Additionally, our data show that (1) ABRs provide a powerful tool to study low-hierarchy probability-encoding by minimally invasive means, (2) differently filtered responses can be utilised to assess different parts of the auditory pathway and (3) bats are an excellent model organism to study deviance detection, due to their evolutionary specialisation towards the auditory domain.

T6.2

Auditory responses of IC neurons in the big brown bat, *Eptesicus fuscus*, during a competitive foraging task

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There is strong evidence that context plays a role in the processing of acoustic signals. Yet, the circuits and mechanisms that govern this process are still not fully understood. *Eptesicus fuscus* bats emit a wide array of communication calls, including food claiming calls, aggressive calls and appeasement calls. Previously, we showed that there are selective neurons for communication and echolocation calls in the inferior colliculus (IC) of *E. fuscus* passively listening to sound playbacks. Now, we developed a novel competitive foraging task to explore the role of behavioral context in auditory responses to social calls. With this approach, we recorded neural population responses from the IC of freely interacting bats. Our data show that bats spend a significant amount of time engaging in interactive social behaviors and emitting communication calls as they compete for food. Furthermore, analysis of our neural recordings from the IC show stronger population responses to individual calls during behaviorally aggressive events. These results indicate that behavioral context plays a role in the modulation of neuronal population responses to social vocalizations in the bat IC.

T6.3

Investigating parallel song memory connections in the zebra finch higher auditory cortex

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Male juvenile zebra finches learn to sing by listening and memorizing, and then mimicking their tutor's song. The caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM) comprise the zebra finch higher auditory cortex. The NCM has been evidenced as a site of tutor song memories (Yanagihara and Yazaki-Sugiyama 2016), while the neighboring CMM has been also reported to correlate with memory formation and directly project to the song motor area, HVC. While it has been hypothesized that the NCM and CMM may act as parallel auditory memory places for tutor song, the connections between these two regions and how the location of tutor song memory may change as birds develop their own song has yet to be understood. Here, by using newly optimized cFos TetON/TetOFF system in zebra finches, we separately labelled the neurons responding to tutor song with EGFP and the ones responding to bird's own song with mRFP (using AAV2/9 cFos-TetON-EGFP and AAV2/9 cFos-TetOFF-mRFP1) in both the NCM and CMM of adult male zebra finches. Anatomical analysis has revealed that distinct neuronal populations were activated by either tutor song, bird's own song, or both in both the NCM and CMM. These populations do not differ in their proportions between the adult NCM and CMM (n=3 birds, $p>0.05$) implying that they are represented equally in both regions in adult male birds. We are also performing tracing analysis of tutor responsive neurons in the NCM which have shown projections to the CMM region and those in the CMM project to the NCM. These suggest that both the NCM and CMM include distinct neuronal substrates of song memories and that those for tutor song memory are interacting with each other.

T6.4

Exploring the role of egocentric movement for shape discrimination during active electrolocation in the weakly electric fish, *Gnathonemus petersii*

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Body movements are known to play an active role in sensing, however, it is not entirely understood what information is provided by these movements. The weakly electric fish *Gnathonemus petersii* senses their environment through active electrolocation during which they perceive object-induced distortions of a self-produced electric field with epidermal electroreceptor organs. The analysis of electric images projected onto their skin enables them to discriminate between three-dimensional objects. While we know the electric image parameters used to encode numerous object properties, we don't fully understand the parameters for shape perception. We hypothesise that 'movement-induced modulations' (MIMs) of the electroreceptive input evoked by body movements might be involved in shape discrimination. MIMs represent the changes that occur to the electric images projected onto the body as a fish swims near an object. Here, we aimed to understand whether body movements are important for shape discrimination during active electrolocation. We trained fish to complete a shape discrimination task in a two-alternative forced-choice setup, and manipulated the space available to individuals for scanning movements to test whether this effected their discrimination performance. We found that if enough space was available, fish were very good at discriminating objects of different shapes. However, performance decreased strongly when the space was reduced and scanning movements were impaired. This shows that the ability to discriminate between shapes is underpinned by the animal's movements. Our study demonstrates the crucial importance of body movements for gaining complex environmental information, such as object shape, through active electrolocation.

T6.5

An electrosensory cocktail party problem

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The "cocktail party problem" is about segregating an auditory stream from background sounds. We studied an electrosensory cocktail party in the weakly electric fish of the species *Apteronotus leptorhynchus* that generate an electric organ discharge (EOD) with a stable frequency. When two fish meet their EODs generate a beating amplitude modulation (AM) with a frequency given by the difference between the two EOD frequencies. When three fish interact a social envelope emerges, with its frequency given by the difference between the two beat frequencies. We studied a situation of three fish, based on observations of resident males that courted females (strong signals) and that simultaneously were able to detect and attack intruder males over large distances (faint signal). How is this faint beat on top of the strong beat encoded and detected by the electrosensory system?
We approached this question with electrophysiological recordings and simulations of realistic leaky integrate-and-fire models of primary sensory afferents, the P-units, that encode AMs in their firing rates. The models revealed nonlinear effects that enhance the response of certain beat frequencies. Still, in the electrophysiological P-unit population the nonlinear effects can be found only in a small subpopulation of the P-units, with a low coefficient of variation (CV) indicating high reliability and low noisiness in the spikes times. These low CV cells might be the basis for an improved detectability of the intruder. An alternative explanation is that nonlinear effects might in fact be detrimental to signal encoding in the P-units. Then the noisiness (high CV) would be an adaptive mechanism in P-units to suppress the undesired nonlinear effects. We discuss these competing hypotheses.

T6.6

The electric ecology of predator-prey interactions: electroreception in caterpillars

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Electroreception, the ability of an organism to detect ecologically relevant electric fields, was historically believed to be restricted exclusively to the aquatic environment. However, recent evidence has emerged demonstrating that bumblebees are capable of detecting the electric fields around flowers, which they may use as a foraging cue. This newly discovered sensory modality, termed 'aerial electroreception', has since been found to be used

similarly by hoverflies to detect floral electric fields, as well as honeybees when detecting conspecifics performing the waggle dance, and in linyphiid spiders detecting the strength of the atmospheric electric fields that provide lift when dispersing by silk ballooning. These discoveries raise the question as to whether aerial electroreception could in fact be a widespread sense amongst terrestrial animals. However, the ecological contexts within which it has been demonstrated thus far are relatively specific behaviors not performed by the vast majority of organisms. As such, it is difficult to claim with confidence that aerial electroreception is common based on these examples alone. To rectify this, it was sought to investigate the possibility that aerial electroreception could be utilised in arguably the most ubiquitous and important ecological interaction of all amongst animals: predator-prey interactions. Here, we present data from behavioural and biophysical experiments showing that caterpillars are capable of detecting the static electricity emitted by their predators, and react defensively when presented with these electrical stimuli. These findings strongly support the hypothesis that aerial electroreception could be a widespread ability amongst terrestrial animals.

PARTICIPANT SYMPOSIUM 7 – CHEMOSENSORY, MECHANOSENSORY AND HYGROSENSORY SYSTEM

T7.1

Investigating odor navigation in *Drosophila* using fictive odor landscapes

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The olfactory system is continuously barraged with a plethora of odors, varying in chemical structure, concentration, and spatial dimensions over different timescales. To navigate within such a complex chemical world, animals must be able to rapidly discriminate between different odors and use spatial signals to localize and track towards an odor source. To gain insight into the core neural and behavioral algorithms underlying olfactory navigation, we have been leveraging the simple architecture of the *Drosophila* olfactory system. We developed a novel closed-loop olfactory system that is compatible with 2-photon imaging that allows us to create fictive odor landscapes with tight spatiotemporal precision, enabling us to directly record from the brain during navigation of an odor plume. Using this system, we found that walking flies track a surface plume using an unexpected but highly robust navigational strategy: rather than walking upwind in the center of an odor plume, flies ascend along its boundary by rapidly counter-turning into and out of the odor. Through optogenetic perturbations we have identified that activity of neurons within brain centers associated with olfactory learning and spatial memory—the mushroom body and central complex—as being essential to this navigational strategy. Using functional imaging, neural perturbations and behavior, we begun to tease apart how these brain centers may contribute to ongoing navigation, allowing a fly to continually evaluate the valence of an odor as it enters the plume or storing a memory about the spatial direction of the plume as it exits. Ultimately, we aim to use this novel behavioral paradigm to elucidate the behavioral algorithms and neural mechanisms of olfactory navigation in *Drosophila.*

T7.2

Chemical signatures of human odour and implications for mosquito olfactory evolution

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Although most mosquitoes are generalists, a few have evolved to specialize on human hosts and thus become major vectors of human disease. These mosquitoes use olfactory cues to target humans, yet we lack an understanding of what makes human odour unique. Here, we undertake the first large-scale survey of vertebrate body odours to discover the chemical compounds employed as host cues by blood-feeding insects and particularly by human specialists. We show that certain compounds are enriched in vertebrate odours and may therefore be used by blood-feeding insects to target hosts. We also show that human odour is unique among animals, even among other great apes, and that these differences probably result from unique aspects of human skin biochemistry. We propose a mechanism by which human-specialist mosquitoes have evolved to cue in on a specific chemical signature of human odour—a mechanism that is consistent with calcium-imaging data. Our work not only has implications for vector control but also demonstrates how understanding the statistics of natural sensory environments can provide novel insight into sensory coding and evolution.

T7.3

Chemical cues mediate mound building behavior in termites

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The mound-building termites *Odontotermes obsesus* build massive, intricate structures out of soil. These mounds, which can attain heights of up to 3 meters above ground, consist of numerous interconnected corridors and galleries leading to the subterranean nesting chambers. The building of these mounds relies on the close coordination of the individual actions of many termites who excavate and deposit soil in the process of construction. How termites communicate with each other to collectively build such large mounds has been a long-standing question. One hypothesis - the so-called stigmergic hypothesis - states that termites communicate with each other by embedding cues in the structures that they build. To test this hypothesis, we devised an experimental assay to determine how termites respond to soil that has been previously processed by other nestmate or non-nestmate termites. In this assay, the termites must choose between two soil patches which are thermally or chemically treated to remove or alter the embedded cues. Our data show that the preference for the soil at the site of repair is mediated by volatile and non-volatile chemical cues deposited by the termites in the soil. The volatile chemicals are short-lived but have a larger spatial range. The non-volatile chemicals, on the other hand, are long-lived and encode a signal that helps termites distinguish between soils handled by nestmates vs non-nestmates. These cues elicit distinct building responses in major and minor workers. Our results suggest that termites communicate with each other by depositing a hierarchical combination of chemicals such that different combinations specify whether a soil patch is being processed by termites that are of the same species, or even from the same nest.

T7.4

Floral humidity as an attractive signal in a nocturnal plant-pollinator interaction

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Although visual and olfactory floral signals attract pollinators from a distance, additional sensory channels often influence pollinator foraging choices at close range. Recent evidence suggests pollinators can utilize floral humidity as an index cue for nectar presence at the threshold of a flower. However, in the night-blooming *Datura wrightii* flowers and their hawkmoth pollinator *Manduca sexta*, we show that *Datura* presents large and sustained humidity gradients in their floral headspace that is not a consequence of nectar evaporation. Datura floral humidity results from gas exchange through floral stomata and is decoupled from nectar presence. We identify the appropriate hygrosensing sensillum on Manduca antennae and show that the underlying neurons are sensitive to minute fluctuations in humidity. Moths show a strong innate attraction to humid artificial flowers over those with ambient humidity and experimental occlusion of the hygrosensing sensillum abolishes this preference. By combining floral physiology, sensory biology, and foraging behavior we present evidence for the function of floral humidity as an attractive signal, not a cue, in this pollination system.

T7.5

Mechanism of touch detection by sensory corpuscles in tactile specialist birds

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The sense of touch is essential for navigation in the environment, object recognition, detection of pain and pleasure. In vertebrates, tactile stimuli are detected by peripheral sensory neurons, but many aspects of sensory physiology remain obscure. To address general mechanisms of somatosensation, we study tactile specialist ducks. The skin covering duck bill contains a high density of mechanosensory corpuscles tuned to detect transient pressure and vibration. These corpuscles comprise a sensory afferent neuron surrounded by lamellar cells. The neuronal afferent is thought to be the mechanical sensor within the corpuscle, whereas the function of lamellar cells is unknown. We developed a preparation of bill skin from tactile-specialist ducks that permits in situ electrophysiological recordings from lamellar cells. We show that lamellar cells within duck Grandry (Meissner) and Herbst (Pacinian) corpuscles detect tactile stimuli via mechanically-gated ion channels, and generate mechanically-evoked action potentials, similar to neuronal mechanoreceptors. These findings provide the first evidence that lamellar cells in mechanosensory corpuscles are active detectors of touch. We propose that Meissner and Pacinian corpuscles use both neuronal and non-neuronal mechanoreception to detect mechanical signals.

T7.6

Unraveling the sensory capabilities of scorpion pectines with a neuroanatomical and behavioural approach

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The chemical sense is an essential and integral modality. In most arthropods, the primary chemosensory organs, the antennae, are associated with the second head segment. However, Arachnida (spiders, scorpions, and relatives) do not possess dedicated chemosensory appendages associated with the second head neuromere but evolved such organs in locations different from the typical mandibulate antennae. In scorpions, the most conspicuous chemosensory organ are the so-called pectines, comb-like appendages located ventrally right behind the fourth walking legs. Besides chemosensation, these organs also detect mechanosensory cues, making them ideal sensors for substrate-borne signals. Despite these fascinating features of pectines, detailed knowledge of their neuroanatomical as well as functional aspects remains rather scarce. We will summarize the neuroanatomical features of the sensory pathway associated with the pectines and will draw comparisons to other arachnids. Although pectines have evolved convergently to other chemosensory organs in arthropods, they possess several striking similarities pointing towards functional prerequisites of such organs (e.g. glomerular neuropil arrangement). However, the pectinal chemosensory pathway shows unique features, suggesting specific adaptations and functions (e.g. a somatotopic representation). We will complete these findings with insights from behavioral assays. Surprisingly, scorpions are able to detect not only nearfield substrate-borne chemosensory cues but also volatile stimuli from distant prey items. These findings re-shape the general opinion that scorpions are solely sit-and-wait-predators and that volatile stimuli play an inferior role in the detection of chemosensory cues in scorpions, and probably also in other Arachnida.

T7.7

Temporal responses of bumblebee gustatory neurons encode sugar identity

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Taste permits the recognition of valuable nutrients and the avoidance of potential toxins. The sense of taste is generally understood to be the intensity-dependent detection of stimuli in a few broad categories (e.g., sweet, bitter, etc.), with little ability to distinguish compounds within a single modality. In *Drosophila*, a single taste sensillum houses between 2-4 gustatory receptor neurons (GRNs), with a single GRN from each sensillum responsive to sugars. Bumblebees rely on sugary nectar as their primary energy source, and they have adapted a specialized taste system for the detection of sugars at the expense of high resolution in other taste modalities. Within a single bumblebee galeal taste sensillum there are four GRNs, and we show that three of these GRNs are responsive to sugars. When stimulated with some sugars, the GRNs fire in a bursting pattern, while other sugars do not elicit bursting at any concentration. A clustering analysis of the temporal responses to sugars over a concentration gradient predicts that bees can distinguish sugars in a few broad categories. We tested this behaviourally and found that bees perform better than the clustering algorithm predicts: bees can distinguish a range of sugars, including those found in nectar (sucrose, fructose, glucose) and honeydew (maltose, melezitose). This suggests that bumblebees have evolved a specialized taste system with high acuity for sugars.

T7.8

Neurobiological mechanisms underpinning behavioural responses to elevated CO_2 in a cephalopod

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The nervous system is central to coordinating behavioural responses to environmental change, likely including rising seawater carbon dioxide (CO_2) levels. We exposed two-toned pygmy squid *Idiosepius pygmaeus* to current-day (~450 µatm) or elevated (~1,000 µatm) CO_2 levels for seven days. Squid were treated with sham, gabazine ($GABA_A$ receptor antagonist) or picrotoxin (antagonist of molluscan GABA-, glutamate-, acetylcholine- and dopamine-gated Cl^- channels) immediately before behavioural measurements, followed by dissection of, and RNA extraction from, the central nervous system (CNS) and eyes. Elevated CO_2 treatment increased conspecific-directed behaviours and activity levels. Both antagonists had different behavioural effects at elevated compared to current-day CO_2 conditions. This supports previous research that disrupted $GABA_A$ receptor function underpins altered behaviour in elevated CO_2 conditions, and provides the first pharmacological evidence, for any marine organism, that other ligand-gated Cl^- channels are also involved in CO_2-induced behavioural changes. RNA-sequencing data from the CNS and eyes was mapped against a *de novo* transcriptome assembly created from PacBio long-read ISO-seq data. We found molecular signatures for disrupted GABAergic, monoaminergic, glutamatergic, and cholinergic neurotransmission, including neurotransmission mediated by ligand-gated Cl^- and cation channels, and G-protein coupled receptors, at elevated CO_2. Furthermore, expression of genes involved in GABAergic and cholinergic neurotransmission were correlated with CO_2-induced behavioural changes in the same individual squid. Our results show that elevated CO_2 disturbs various types of neurotransmission, thereby altering vital ecological behaviours.

PARTICIPANT SYMPOSIUM 8 – VISUAL SYSTEM II

T8.1

Short-term plasticity of the *Amphiprion ocellaris* visual system in response to anthropogenic changes to the light environment

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Coral reef fishes are exposed to 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 indicated that some reef fishes can alter their visual (opsin) gene expression under different light conditions. However, whether and how changes in gene expression translate to visual performance remains untested. In this study, we examined the capacity of the ocellaris clownfish (*Amphiprion ocellaris*) visual system to adapt to short-term light environment changes. The chosen induced light environments reflect ecologically relevant stressors found on the Great Barrier Reef: enhanced sediment runoff and algal blooms. Firstly, we showed the ability of adult *A. ocellaris* to shift their opsin expression within 10 days of having a different light environment. Secondly, we used behavioural experiments to explore if the changes at a molecular level would alter the threshold at which two colours can behaviourally be distinguished. We found that the largest changes in opsin expression and colour discrimination thresholds occurred in the green algal-simulated light environment. Overall, our results show the potential for short-term plasticity both on a molecular and behavioural level and thus clownfish likely have the capacity to quickly alleviate the effects of environmental change.

T8.2

Visually-guided proboscis movements fine-tune flower probing in the hummingbird hawkmoth

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Visually-guided reaching – to your coffee cup or a door handle – is a central feature of primate behaviour. In insects, however, limb movements often follow a stereotyped motor programme. Only a few instances of visual appendage guidance are known. These are the forelimb reaching movements of horse-headed grasshoppers and fruit flies during gap crossing (Triphan et al. (2010) *Curr. Biol*. 20: 663-668, Niven et al. (2012) *Proc. R. Soc. B*. 279: 3697-3705). Yet, while these species adjust the amplitude of the reaching movement to gap size upon initiation, there is no evidence that visual feedback updates the ongoing limb movement, as is the case when primates reach for a goal (Hayashiet al. (2016) *eNeuro*, 3:ENEURO.0032–16.2016). Here we provide evidence for an insect using visual feedback for appendage control: the targeted proboscis probing on visual flower patterns by the hummingbird hawkmoth (*Macroglossum stellatarum*). To study these proboscis movements, we filmed hawkmoths at high speed while they inspected artificial flowers with colourful patterns. To separate the contributions of flight control that passively positions the proboscis on the flower from active proboscis control, we restricted the animals' body movements relative to different pattern orientations. We thus demonstrate that hawkmoths fine-tune their proboscis placements visually, in addition to the coarse control provided by flight movements. By selectively occluding the hawkmoths' frontal visual field, we furthermore demonstrate that this proboscis guidance requires continuous visual feedback on the proboscis position on the pattern. Together, our results provide the first evidence for visually-guided proboscis control in insects.

T8.3

The colourful retinal mosaic of nymphalid butterflies

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Brush-footed butterflies (Nymphalidae) have colour vision ranging from the UV to the red part of the light spectrum. Their compound eyes are built of thousands of ommatidia, each housing nine photoreceptors. Incident illumination displays a facet reflection pattern, the eye shine, which is uniform or complex, dependent on the species. Facet colours suggest that the ommatidia contain different sets of photoreceptors and screening pigments, but how the colours and the cell characteristics are associated has not been clearly established. We have analysed the retinae of ten nymphalid species from genera *Archaeoprepona, Argynnis, Charaxes, Danaus, Melitaea, Morpho, Heliconius, Vanessa* and *Speyeria*, using single-cell recordings, spectroscopy and optical pupillometry. The species with a homogeneous eye shine have UV, blue and green-sensitive photoreceptors, allocated into three ommatidial types. The UV and blue-sensitive cells are the long visual fibres, receiving opponent input from the green-sensitive short visual fibres. The species with a complex eye shine have an expanded set of photoreceptors, allocated into three additional, red-reflecting ommatidial types. The red colour is due to the screening pigment, apposed to the rhabdom, which filters the incident light and creates several additional photoreceptor types. All red ommatidia contain green-sensitive LVFs, receiving opponent input from red receptors. The red receptors are the tiny, basal cells R9. The simple retina with three ommatidial types and two colour-opponent channels can support trichromatic vision, while the complex retina with six ommatidial types and three colour-opponent channels can support tetrachromatic vision.

T8.4

Persistent angular velocity bias after wide field visual motion presentation in flying *Drosophila*

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In bilaterally symmetric animals, differences between the two sides of the body across the midsagittal plane can produce systematic locomotory deviations to one side. In such cases, animals use visual feedback to calibrate their motor output to compensate for the locomotory bias. To investigate this form of motor system flexibility, we examined the flight behavior of tethered *Drosophila melanogaster* flies in a virtual reality arena where flies are able to freely rotate in yaw. To mimic a locomotory bias produced by body asymmetry, we exposed flies to wide-field visual motion in the yaw direction. As expected, presentation of wide-field yaw stimulus induced the characteristic optomotor response, in which flies maintained a constant angular velocity to follow the visual pattern motion. In other words, the flies altered their motor output to compensate for a perceived locomotory bias. When the visual stimulus was then removed, and flies were left to fly in complete darkness, flies maintained the elicited angular velocity for up to several minutes, suggesting they can maintain this recalibrated motor output, or trim, without relying on external cues. Subsequent experiments showed the magnitude of the flies' angular velocity during the period of darkness was correlated to the duration of the visual presentation. Even brief (3s) wide field motion presentations biased the flies' angular velocity for at least a minute during the subsequent period of darkness. Having established this system to study motor system flexibility in the genetically tractable fly, we are now well positioned to investigate the neural systems that detect, produce, and maintain this locomotory bias.

T8.5

Nocturnal flying insects are trapped by illumination due to their dorsal-light-response

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For as long as humans have created artificial light during the hours of darkness, nocturnally active insects have appeared drawn to the light. This effect is so overpowering that light-traps became a well-established means of assessing insects in the field. Theories as to why insects appear drawn to light cover a wide range of potential mechanisms, from emergency escape responses to confusion of a lunar celestial compass. The extensive number of theories is contrasted by a lack of high temporally and spatially resolved behavioural data. To evaluate these theories, we used a combination of micro-motion capture in the lab and high-speed stereo-videography in the field to reconstruct flying kinematics of nocturnally flying insects around artificial light sources. In our recordings, insects neither flew directly toward a light source, nor followed the 'log-spiral' attraction predicted by a lunar compass model. Instead, insects entered a variety of unstable flight attitudes as they directed their dorsum toward the light. This is indicative of the well known dorsal-light-response detailed in diverse insect taxa as a key in-flight orienting mechanism. Dorsal tilting toward light is a highly conserved stability mechanism for insect flight, explaining the great sweep of taxa that appear drawn to nocturnal illumination. However, differences in flight kinematics led to different steering responses, and ultimately to different flight trajectories. Our findings

↑UP

not only suggest a parsimonious mechanism for nocturnal light entrapment, but also a new avenue into assessing differences in insect flight control.

T8.6

Spatial resolution and optical sensitivity in the compound eyes of two common wasps, *Vespula germanica* and *Vespula vulgaris*

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Vespula germanica and *Vespula vulgaris* are two common European wasps that have ecological and economic importance due to their invasive introduction to many different countries and environments. Their success as organisms has been enabled partly by their vision, their visual behaviour and their capacity to learn visual cues in the context of homing and navigation. In general, diurnal hymenopterans are well known for their ability to memorise "visual snapshots" of landmarks along their foraging routes in order to orient themselves and to find their way back to the nest after foraging trips. However, the visual systems of *V. germanica* and *V. vulgaris* have not received any deep attention. We used electrophysiology, together with optical and anatomical techniques, to measure the spatial resolution and optical sensitivity of the compound eyes of both species. We found that both wasps have high anatomical spatial resolution with narrow interommatidial angles and a distinct acute zone in the frontal-ventral part of the eye (2.0° for *V. germanica* and 2.2° for *V. vulgaris*). These narrow interommatidial angles are matched to photoreceptors having small angular sensitivities, indicating eyes of high spatial resolution well suited to their ecological needs. Additionally, we found that both species exhibit an optical sensitivity that is typical of other day-flying hymenopterans.

T8.7

Parallel spatial 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. This complex sensory input requires a high amount of flexibility in the visual system (Bigge et al., 2021, Curr Biol). Insects in particular, due to their limited neural capacities, need to streamline the visual input signals. An important neuropil for such filtering is the lamina, the first visual processing stage. Its main relay neurons, lamina monopolar cells (LMCs), receive information directly from the photoreceptors. Recently, we have shown that one type of LMC dynamically adjusts its sensitivity and spatial tuning with light intensity in the hummingbird hawkmoth *Macroglossum stellatarum.* To this aim it integrates visual information via its lateral processes that reach into neighboring visual units, called cartridges (Stöckl et al., 2020, Sci Adv). To assess the spatial processing potential of the other LMC types in the hawkmoth lamina, we used serial block-face scanning electron microscopy to obtain a high-resolution image stack through the entire depth of the lamina. Reconstructing the fine-structure of all -the cells within one cartridge revealed new cell types which have not been described in previous Golgi studies. Based on our findings, we suggest a novel classification of LMC types, coordinated with recent findings in other insect groups. To predict the LMCs spatial processing potential, we labelled the synaptic connections of the reconstructed neurons. Combining this anatomically-based model with novel data on the cells' physiological properties provides a mechanistic explanation for how lamina neurons process complex spatial input in parallel spatial channels, as well as dynamically across light intensities.--

↑UP

T8.8

Characterization of wide-field motion sensitive neurons in the central brain of the bumblebee

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Moving animals experience wide-field motion due to the displacement of the retinal image. These cues provide information about rotatory and translational self-motion (Gibson, 1950) and allow to estimate different parameters like flight speed (David, 1982) or distance (Esch and Burns, 1995). They are also involved in more complex tasks like path integration for which an odometer is indispensable (Wehner and Srinivasan, 2003). Input neurons to the noduli of the central complex (TN-cells) encode translational optic flow and are thought to provide distance information for path integration (Stone et al., 2017). Here we investigated tuning properties of motion-sensitive neurons in the noduli of the bumblebee by presenting moving stripe patterns at different temporal and spatial frequencies. We conducted extracellular tetrode recordings in tethered animals. Electrodes were dipped in fluorescent dye to confirm the electrode position near the noduli using confocal microscopy. Response characteristics of units in our recordings matched the ones for TN-cells described in Stone et al. (2017). We were able to identify tuning properties of TN-cells to wide-field motion responding excitatory to either simulated forward or backwards flight and inhibitory to the respective other direction. Our results show that units were tuned to temporal frequencies between 10 and 20 Hz independent of spatial frequency. Nevertheless, tuning curves for spatial frequencies differed in spike rate. This indicates that spatial frequency provides information for the encoding of wide-field motion, too. The combination of temporal and spatial frequency could provide an estimate for travel distance. This suggests that response properties of TN-cells could indeed serve as an odometer for path integration.

PLENARY SESSION 9

The gourmet fly: dissecting the mechanisms underlying feeding decisions

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A balanced intake of different classes of nutrients is a key determinant of health, wellbeing, and aging. To ensure nutrient homeostasis animals adapt their foraging strategies according to their current and future needs. We want to understand how animals decide what to eat, how these decisions are shaped by brain-body interactions, and how these decisions affect the fitness of the animal. To achieve a mechanistic, integrated, whole-animal understanding of nutritional decision-making we work at the interface of behavior, metabolism, microbiome, and physiology in the adult Drosophila melanogaster. I will discuss how the powerful combination of activity imaging approaches, neurogenetics, automated, quantitative behavioral analyses, nutritional and microbial manipulations, and metabolomics is allowing us to achieve a mechanistic understanding of how internal states shape neuronal circuits to optimize complex foraging decisions.

PARTICIPANT SYMPOSIUM 9 – SPATIAL ORIENTATION AND NAVIGATION II

T9.1

Source identity shapes spatial preference in primary auditory cortex during active navigation

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Information about the position of sensory objects and identifying their concurrent behavioral relevance is vital to navigate the environment. In the auditory system, spatial information is computed in the brain based on the position of the sound source relative to the observer and thus assumed to be egocentric throughout the auditory pathway. This assumption is largely based on studies conducted in either anesthetized or head-fixed and passively listening animals, thus lacking self-motion and selective listening. Yet these factors are fundamental components of natural sensing that may crucially impact the nature of spatial coding and sensory object representation. How individual objects are neuronally represented during unrestricted self-motion and active sensing remains mostly unexplored. Here, we trained gerbils on a behavioral foraging paradigm that required localization and identification of sound sources during free navigation. Chronic tetrode recordings in primary auditory cortex during task performance revealed previously unreported sensory object representations. Strikingly, the egocentric angle preference of the majority of spatially sensitive neurons changed significantly depending on the task-specific identity (outcome association) of the sound source. Spatial tuning also exhibited large temporal complexity. Moreover, we encountered egocentrically untuned neurons whose response magnitude differed between source identities. Using a neural network decoder, we show that, together, these neuronal response ensembles provide spatiotemporally co-existent information about both the egocentric location and the identity of individual sensory objects during self-motion, revealing a novel cortical computation principle for naturalistic sensing.

T9.2

Neural representation of 3D space in the freely navigating goldfish by axial encoding schematics

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For animals, the ability to situate themselves in the environment can be crucial for survival. Although present in all vertebrates, research has focused on rodents and mammals navigating specifically in 2D environments. We lack insight into the neural representation of 3D space in fish, the largest vertebrate class. To address this issue, we used wireless technology to record the activity of single neurons in the central area of the goldfish telencephalon while the fish freely navigated in a 3D water tank. We found cells tuned to the location of the fish along different axes of space. Of these cells, some were tuned along the principal cartesian axes: horizontal and vertical, while others were tuned to position along an axis from a salient feature of the environment. This type of axial code for space representation in the brains of fish is unique among the space encoding cells in vertebrates and provides insights into spatial cognition in this lineage.

T9.3

Screening for magnetically induced neuronal activity in the pigeon brain

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The rock pigeon (Columba livia) is a navigation specialist with the ability to detect magnetic fields. Despite compelling behavioral evidence demonstrating the existence of a magnetic sense in birds, the underlying neuronal circuits dedicated for processing magnetic information are largely unknown. To address this, we exposed head-fixed pigeons to a rotating magnetic stimulus in the dark and used tissue clearing and light sheet microscopy to screen the whole pigeon brain for neuronal activity. To globally assess magnetically induced effects, we created a custom pigeon brain reference template for anatomical mapping of individual brain scans and adapted the open source software ClearMap to quantitate the number of activated neurons in birds exposed to magnetic fields versus non-exposed birds. These experiments revealed a magnetosensory circuit that involves the medial vestibular nuclei in the brainstem, the midbrain dorsal thalamus and forebrain regions including the mesopallium and hippocampus. In the future we anticipate that this pipeline will provide key insights into the neuronal networks underlying navigation in a broad range of species during ethologically relevant contexts.

↑UP

T9.4

Neuromorphic mushroom body model learning outdoor routes in real-time

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Biologically inspired robots are well suited to link the evaluation of biological hypotheses with novel solution of engineering problems. Insects are expert navigators although it is unclear how they encode visual motion by their miniaturised brains and relatively simple neural systems. Work in robotics, e.g., SeqSLAM, has alternatively shown that matching the sequence information in video frames during repeated traversals of a route can significantly improve the ability to localise the current position on the route. In this work we built a hardware robot embedded with bio-inspired event camera and mushroom body (MB) spiking neural network (SNN), and tested visual motion learning in outdoor natural environments. Unlike previous mushroom body models where visual snapshot patterns are learned by reducing Kenyon cell (KC) to output neuron weights with all temporal information discarded, here we learn continuous visual motion flow pattern captured by an event camera during robot movement. We postulated that the newly found but functionally unexplained KC-KC interconnections can encode visual motion pattern into spatial-temporal memory. The sensing method, neural network structure, basic neuron models and the learning rule in our system are all biologically constrained and plausible. Compared to SeqSLAM, our model is more sensitive to input changes and perform better in offset route recognition. By encoding memory into an SNN and running it on a neuromorphic computer, our model can perform real-time route recognition without storing global maps or retrieving visual patterns.

T9.5

From fish on dry land to new insights on navigation mechanisms in animals

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Navigation is a critical ability for animal survival it is important for finding shelter, seeking mates, food foraging, and a variety of other behaviors. Given their fundamental role and universal function in the animal kingdom, it makes sense to explore whether navigation mechanisms and space representation are dependent on the species, brain structures, ecological system, or whether they share general and universal properties. One way to explore this issue behaviourally is by *domain transfer methodology*, where one species is embedded in another species' environment and must cope with an otherwise familiar (in our case, navigation) task. We push this idea to the limit by studying the navigation ability of a fish in a terrestrial environment. For this purpose, we trained goldfish to use a Fish Operated Vehicle (FOV), a wheeled terrestrial platform that reacts to the fish's location, orientation in the water tank, and movement characteristics to change the vehicle's; i.e., the water tank's, position in the arena. The fish were tasked to "drive" the FOV towards a visual target in the terrestrial environment, observable through the walls of the tank. Indeed, the fish were able to operate the vehicle, explore the new environment, and reach the target regardless of the starting point, all while correcting location inaccuracies and avoiding dead-ends. These results demonstrate how fish were able to transfer their space representation and navigation skills to a wholly different terrestrial environment, thus supporting the hypothesis that the former possess a universal quality that is species-independent.

T9.6

Magnetic pulses as a directional assay for studying magnetoreception in the Caribbean spiny lobster

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Even with decades of evidence that phylogenetically diverse animals sense Earth's magnetic field and use it to navigate, no primary magnetoreceptor has been identified in any animal. Therefore, magnetoreception research

has often focused on assays that would theoretically interfere with proposed transduction mechanisms. In the case of a magnetite-based transduction mechanism, this has taken the form of applying a strong, transient magnetic field known as a magnetic pulse to the animal being studied. Theoretically, such a pulse would remagnetize magnetic material—and thus disrupt orientation behavior—without affecting other proposed transduction mechanisms. In previous work, we proposed that because this magnetic pulse necessarily has an orientation, one might be able to use it as a directional (rather than binary) assay. In other words, one might deduce organizational or computational properties of an animal's magnetoreceptive system via studying its responses to differently directed pulses. Using the results of a prior study in the Caribbean spiny lobster *Panulirus argus*, we were able to construct neural models of magnetoreception transduction and processing that responded similarly to previously applied pulses. Groups of simulated and real lobsters tended to travel in similar directions when subjected to the same magnetic pulse. However, because these models were made to explain behavior we had already observed, the best test of their accuracy is to assess their ability to predict future behavior. Here, we close the loop of theory and behavior by testing our computational model's ability to predict lobsters' responses to novel pulse directions. In so doing, we develop and test a falsifiable hypothesis of *P. argus* magnetoreception transduction and processing.

PARTICIPANT SYMPOSIUM 10 – COMMUNICATION, SOCIAL BEHAVIOR AND BRAIN

T10.1

The neuroethology of vocal communication in zebra finches: Perception of an entire vocal repertoire.

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Vocal communication in oscines is impressive, not only because of the flexible production and usage of song but also because of the number of vocalization types in their repertoire that support a diversity of social interactions. In the last ten years, we have studied the mechanisms involved in the perception of all call-types in the vocal repertoire of the zebra finch. We have performed bio-acoustical, behavioral, neurophysiological and computational analyses to decipher the acoustic and neural code involved in this sound to meaning transformation. Zebra finches use 10 ethologically defined call-types used for social bonding and to communicate aggression or distress. The acoustic code for these call-types relies on a mixture of discrete and graded signaling. Nonetheless, we found that small ensembles of neurons in the auditory pallium can effectively categorize all call-types. Ensemble neural discrimination was not correlated with single neuron selectivity, but instead with how well the joint single neuron tunings to spectro-temporal modulations spanned an acoustic subspace optimized for the discrimination of call-types. Computational modeling shows that the tuning for these high-level auditory features can emerge in an unsupervised manner by simply attempting to maximally discriminate each call rendition from all other call renditions. Thus, call-type recognition in the auditory system is based on a sparse code representing a small number of high-level features, and not on highly selective grandmother neurons. This efficient code can be learned by simple exposure to the statistical structure of all calls in the vocal repertoire.

T10.2

Impact of informational masking on the acoustic communication of frogs

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Noise is ubiquitous and has profound impacts on acoustic communication in humans and other animals. Perception of vocal signals is often impaired in high background noise. In animal communication studies,

impaired perception in the presence of noise is mostly attributed to energetic masking, which occurs when signal and noise produce competing excitation in the same peripheral auditory filter. Studies on the human auditory system, however, have determined that our ability to perceive speech in noise is also limited by the information processing capacity of the central auditory system. Surprisingly, this so-called problem of "informational masking" has not been investigated in the context of animal communication, even though animals frequently need to extract information from vocalizations in noisy social environments to perform biological functions. We investigated the impact of informational masking on auditory perception in Cope's gray treefrogs (*Hyla chrysoscelis*), a species that communicates in noisy breeding choruses. Using phonotaxis experiments, we found that females were less likely to respond when the simulated mating calls of males were presented concurrently with other biotic sounds designed to cause minimal energetic masking. Additionally, we found this effect of informational masking to be especially strong when competing sounds were present in a specific frequency range. These data provide evidence that informational masking can impair auditory perception in non-human animals and highlight how noise may interfere with central processing to constrain animal communication.

T10.3

Vision and vocal communication guide 3-D bird flock formation during flight

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Animal collective motion is a natural phenomenon readily observable in various taxa. Although theoretical models can predict the macroscopic pattern of group movements based on the relative spatial position of group members, it is poorly understood how group members exchange directional information that enables the interindividual spatial coordination during collective motion. Although in flying groups of birds, vision has been suggested to play a major role in interindividual spatial coordination, many avian species also vocalize during flocking flight. To test if these vocalizations are used by birds to transmit directional information between group members, we recorded vocal behavior, head orientation and spatial position of each individual in a small flock of zebra finches (*Taeniopygia guttata*) flying in a wind tunnel. We found that the birds use visual and acoustic cues to coordinate their spatial positions within the flying flock. While zebra finches always pointed their gaze in the direction of position change when moving in horizontal direction, calls emitted during flight were significantly correlated with upwards directed position changes of the calling bird. When light levels were reduced during flight, birds increasingly exploited active vocal communication to avoid collisions with flock mates. However, when the birds were prevented from communicating vocally, collision rates in the flying flock increased. Our study furthers the mechanistic understanding of collective motion in birds and highlights the impact interindividual vocal interactions can have on group performances.

T10.4

Testing the social brain hypothesis in the wild: how increasing social complexity relates to behavioural repertoire size and neuroanatomy in the Lake Tanganyikan cichlid radiation

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The social brain hypothesis posits that social living has led to selection for increased brain size, but there are alternative ecological explanations for differences in neuroanatomy. Here we take a comprehensive approach to examine the relationship between social complexity, behavioural repertoire size and neuroanatomy, going beyond metrics of overall brain volume to more detailed studies of neuroanatomical structure. We use the shell-dwelling cichlids of Lake Tanganyika, a group of species that inhabit the same ecological niche, have similar diets, have similar life-histories, similar body sizes and morphologies, and live in overlapping, mosaic communities. Thus any confounding factors of ecology or life-history are minimized, while still showing strong variation in the trait of interest: social organization. For 120 wild individuals across 6 species , we employed a combination of

automated tracking of interactions, social network analyses, and behavioural scoring to create individual-level metrics of social context and behavioural repertoire size. Social complexity was calculated per individual based on the number of differentiated interactions with social partners, and repertoire size as the number of distinct behaviours. We compared these metrics against neuroanatomy, finding that with increasing social complexity, species-level behavioural repertoire size increases and that both total brain weight and volume, as well as that of brain sub-regions including the telencephalon and optic tectum, broadly correlated with a species' social complexity, whereas other regions including the cerebellum did not. Overall, these results support the hypothesis that increasing social complexity leads to increased investment into brain regions associated with social behaviour.

T10.5

Neural codes for natural social behaviours in a bat colony

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Highly-social animals live in complex communities and interact with many other individuals – at times, locations and manner of their choosing. However, neurophysiological investigations of social responses are rarely conducted in rich multi-animal settings that allow such natural behaviours. To understand how the brain – and specifically the hippocampus – represents such natural social information, we established a mixed-sex colony of 5–10 Egyptian fruit bats living 24×7 in a laboratory-based "bat cave". We tracked the behaviour of all the bats using a set of high-resolution cameras and radiofrequency-based positional tracking, and simultaneously conducted wireless neural recordings in 1–2 bats for several hours every day to investigate how male and female bats represent social information – when the behaviour of the animal is naturalistic and not experimentally constrained. We found that the behaviour comprised of three distinct and interleaved phases: (i) Flight phase – where we found that dorsal hippocampal CA1 neurons exhibited social and identity coding. (ii) Social Interaction phase – where a subset of hippocampal neurons represented specific social interactions (e.g. allogrooming or aggression). (iii) Sedentary phase – where we utilized generalized additive models (a nonlinear extension of GLM) and explainable machine learning methodologies (like Shapley values) – and found that hippocampal neurons simultaneously represented information related to both self and others, with some neurons encoding a few behavioral dimensions while other neurons encoded many dimensions. Overall, we found that hippocampal dorsal CA1 neurons combine complex social and spatial information to form a multidimensional representation of the natural world.

T10.6

Social modulation of the gut-brain axis in crayfish

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Although the mammalian gut-brain axis has been identified as a modulator of brain health, the neurobehavioral mechanisms remain elusive. Our lab uses crayfish as a non-traditional model system to study the bidirectional pathway between the gut and central nervous system (CNS). We first sequenced the gut microbiota of crayfish that were housed individually or in pairs for 10 days, to better understand the effects of social environment on the gut microbiome. Interestingly, we found a higher abundance of pathogenic bacteria in isolates, suggesting that social deprivation may weaken gut health and its immune defense, and this effect was most pronounced in females. To explore socially-mediated changes of the gut microbiome, we investigated the role of the crayfish CNS in regulating gut behavior. Eliminating CNS inputs to the hindgut in an *ex vivo* preparation altered several features of gut motility, including contraction amplitudes and frequencies as well as the directionality of wave propagation. The neurotransmitter serotonin (5-HT), the most common neurochemical of the mammalian enteric nervous system, was able to restore contraction amplitudes in a denervated gut, but failed to replicate the finely-tuned movement patterns observed in an intact gut-CNS preparation. Together, this suggests that gut motility is centrally regulated in crayfish and modulated in part by 5-HT. Our current approach focuses on neural signals that originate in the hindgut and project to the CNS via a single intestinal nerve. Using combination of electrophysiology and neuropharmacology, we explore how gut-derived neural activity shapes CNS circuits that produce adaptive motor outputs, with the long-term goal to fully uncover the link between social experience, gut-brain axis, and natural behavior.

PARTICIPANT SYMPOSIUM 11 – EVOLUTION AND DEVELOPMENT

T11.1

Transition of neural activities during the development of *Ciona* swimming CPG

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According to the Half Center Model (Brown, 1911), Central Pattern Generator (CPG) of vertebrates are constituted by motor neurons (MNs), interneurons (INs), and ascending contralateral inhibitory neurons (ACINs). However, it is yet unclear how the neural activities of these components are developed during their embryogenesis. Our previous study revealed that in *Ciona robusta*, a model organism with a simple neural circuit, a single pair of MNs (MN2L/MN2R) were determining the rhythm of its spontaneous early motor behavior (developmental stage St.22-24) (Akahoshi *et al.*, 2021). MN2s are known to be one of the main components of *Ciona* CPG (Horie *et al*., 2010), though the neural activities of MN2s during its swimming behavior (St.25-) were not yet investigated. In this study, we investigated the neural activities of MN2s during its later stages and how they are related to *Ciona*'s swimming CPG. Long-term simultaneous Ca^{2+} imaging of MN2s with GCaMP6s/f (St.22-34) revealed that their activities could be classified into 7 phases (I-VI), depending on the interval and synchronicity of MN2L and MN2R Ca^{2+} transients. At first each MN2 oscillate sporadically (I), and as they develop to swimming larva, they gradually oscillate at a constant interval (II-III'), synchronize (IV), oscillate at longer interval (V) and becomes sporadic again (VI). Interestingly, 76% of the embryos started to oscillate from MN2R. Furthermore, contralateral axons of ACIN1s elongated between III' and IV. Optogenetic approaches by miniSOG2/ChR2, and membrane potential imaging of MN2s and ACIN1s supported the idea that ACIN1s are contributing to the rhythmical left-right alternate inhibition of tail muscles. This is the first report of the live imaging of neural activities in *Ciona*'s developing swimming CPG.

T11.2

Complexity of social environment during development affects neural and social behaviour phenotypes in adult zebrafish

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The social environment of an animal can have a profound and determining effect on its behaviour. During development, variations in the social structure provide inadvertent information that influences many aspects of individual development on a wide range of physiological and cognitive traits. In the present work, we studied the influence of environmental complexity in the development of different adult social phenotypes by combining the study of gene expression, neuron numbers and behaviour. Therefore, zebrafish were raised in social environments with different complexities, by varying group size and group stability, until adulthood and then tested in different behavioural tests. Our results indicate that group size and group stability influence differentially adult social behaviour, namely, group cohesion is influenced by group size, whereas social preference responds to group stability. Moreover, animals raised in less complex social environments experience changes in neuronal densities and a significant reduction of volume in specific brain regions related to social information processing. Differences in the transcriptional profiles were also found between treatments, indicating distinct regulation patterns. Together, these results indicate that social complexity during development can significantly impact adult neural and social behavior phenotypes.

T11.3

Dynamic evolution of diel activity patterns across over 400 million years of fish evolution

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Many species are active during different periods of the diel cycle (e.g. day vs night), affecting their ecologies and coexistence with other species. The last common ancestor of Tetrapods was likely nocturnal, and the evolution of diurnality has been associated with increased diversification rates and ecological niche expansion. However, little is known about the evolutionary history, lability, and ecological correlates of temporal partitioning in fishes, the sister lineage to tetrapods. To address this gap in knowledge, we assembled a dataset of temporal activity patterns for > 2000 species of fish, covering over 400 millions years of evolution. We found a strong phylogenetic signal for temporal niche, and that the most likely ancestral state was nocturnal, with roughly equal numbers of extant diurnal and nocturnal lineages. Comparisons to tetrapod lineages revealed that the temporal niches of fish lineages are remarkably labile, with over 800 independent transitions between nocturnality and diurnality (and back to nocturnality) on the fish tree alone. Clades within percomorpha showed highly variable diel activity patterns, including *Perciformes*, *Syngnathiformes*, and *Gobiiformes.* This extreme mutability in diel activity is also demonstrated by our thorough behavioural screen of diel patterns in the ~10my Lake Tanganyikan cichlid radiation (see our other abstract Nichols and Shafer et al). Analysis of ecological traits revealed a high overlap between nocturnal and diurnal lineages, suggesting extensive temporal niche partitioning rather than obligate ecological and temporal niche relationships. Our results reveal previously unrecognised temporal niche diversity in fishes, which has contributed to their extensive diversification rates and evolutionary success.

T11.4

Seeing the world in a new light: Fan worms travel a unique evolutionary path to vision

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Fan worms (Sabellida) are sedentary aquatic polychaetes that reside within protective tubes in the substrate. They extend a crown of radiolar tentacles into the water column from their heads for feeding and respiration. Fan worms rely on sensory structures on the radioles to detect threats and trigger a rapid retraction. In many species these structures include ocelli or compound eyes. These eyes are notable for their independent evolutionary derivation and for possessing widely varying structures and arrangements in different species. While many species have dozens of compound eyes scattered across all of their tentacles, one genus has evolved a single pair of large, consolidated compound eyes with over 1,200 facets, making this an ideal group in which to study the transition between distributed and centralized visual systems and the synthesis of increasingly-sophisticated visual capabilities. We used laboratory behavioral experiments in order to probe the spatial resolution, contrast sensitivity, and motion detection thresholds of two species of fan worms; one with distributed eyes, *Bispira guineensis*, and one with a pair of large consolidated eyes, *Acromegalomma vesiculosum*. We compared these results with 3D models of their visual systems derived from synchrotron µCT reconstructions, ERGs, and environmental light data to illuminate the functional basis of the fan worm visual system. We found that large consolidated eyes offer considerable advantages in the accurate detection of threats, and may indicate that this species has evolved an alternate form of spatial vision through a unique behavioral continuity. Future research into the neural processing circuits for these eyes will yield insights into neural adaptation that animals utilize to achieve spatial vision.

↑UP

T11.5

Function and evolution of high-resolution spatial vision in conch snails

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All species within the conch snail family Strombidae possess camera-type eyes of surprising size and sophistication compared to those of other gastropods. Although strombid eyes are known to be structurally complex, very little research on their visual function has been conducted. Here, we use isoluminant looming visual stimuli to test for behavioural evidence of high spatial resolution in a strombid, *Conomurex luhuanus*. Using these stimuli, we show that this species responds to objects as small as 1° in its visual field, which is finer resolution vision than the majority of invertebrate species. For example, this resolution is similar to that of a worker bee which uses vision for complex flight maneuvers. Although perhaps surprising in a non-predatory snail, these findings are consistent with calculations of spatial resolution based on histological data. We also use serial block-face scanning electron microscopy to reconstruct the complexity of the retina structure which gives rise to this high-resolution vision. We compare these results to estimates of spatial resolution for the eyes of other families within the superfamily Stromboidea, of which, three families have much smaller eyes than *C. luhuanus*, and a spatial resolution about five times coarser for specimens examined. This disparity in visual capabilities and eye structures within one superfamily raises exciting new questions about why strombids have such fine spatial resolution. These results also demonstrate Stromboidea to be an excellent group for studying trends in eye evolution, which we which we are investigating by integrating morphological and genetic data within a phylogenetic context.

T11.6

Visual specialisation and explosive expansion of the mushroom bodies in *Helcionius butterflies*

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Neural innovation, elaboration and refinement underpins behavioural diversity. Yet our understanding of how neural systems evolve, and the mechanisms that govern links between brain and behavioural change remain underdeveloped. We describe a dramatic example of neural elaboration, linked to the evolution of a novel behaviour. *Heliconius* are the only butterflies known to actively digest and collect pollen. This dietary innovation is linked to more restricted individual home ranges, and a spatially and temporally faithful foraging behaviour that is reliant on visual cues. We show that across the wider tribe of Heliconiini, the mushroom bodies, a major insect learning and memory centre, vary in raw volume by over 25-fold. Rapid bursts in mushroom body size occurred in multiple branches of the Heliconiini, but the largest coincides with the origin of pollen feeding and, even after accounting for brain size, results in a doubling of MB size at the base of *Heliconius*. This volumetric expansion is driven by concomitant increases in intrinsic MB neurons, but consistent scaling of MB components and synaptic densities. Strikingly, much of this variation can be linked to increased visual input to the MB in *Heliconius*, altering the dominant sensory modality processed by the MB. We further show that MB expansion is accompanied by increased performance in multiple, visually based cognitive assays, consistent with theoretical expectations that cognitive capacity increases with MB size. Our data highlight Heliconiini as a tractable system for comparative, detail-rich analyses aimed at understanding the adaptive and mechanistic basis of neural and behavioural evolution.

↑UP

PARTICIPANT SYMPOSIUM 12 – LEARNING, MEMORY AND COGNITION II

T12.1

Neuronal activity of mushroom body extrinsic neurons during visual differential learning in honey bees

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The visual learning behavior of free-flying honey bees has been profusely investigated through conditioning to multiple types of stimuli. Bees can learn to differentiate colors and shapes as well as learn several forms of non-elemental visual learning. However, the brain mechanisms underlying these performances have remained in part elusive due to the use of freely flying animals and because restricting the animal's behavior to investigate neural activity would tend to hinder visual learning. Here we aimed to overcome this problem by developing a behavioral protocol for conditioning harnessed bees to discriminate visual stimuli through many successive trials while recording neural activity. Harnessed bees were conditioned to discriminate a rewarded (CS+) from a non-rewarded (CS-) color ring differing in color and moving towards the fixed animals automatically. We performed extracellular recordings of mushroom body output neurons at a single-unit level along trials of a pre-test, a training phase and a subsequent test. At the same time, we recorded the M17 muscle which provides an accurate readout of proboscis extension reflex (PER). We showed that bees learned the discrimination tasks and that the neuronal units recorded varied in their response pattern, some being predictive of CS+ responses and other showing combination of responses varying in time. In most cases the response pattern changed drastically when a sucrose reward was presented for the first time with the color stimulation. This initial change was independent of the color, which would change over trials until the differential response to the CS+ could be observed via PER and a stable spike pattern that would differ between rewarded and non-rewarded developed.

T12.2

Exploring the inter-individual variability in cognitive performance of honeybees

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Although bees have imposed themselves as the main cognitive model within Invertebrates, variabilities in individual performances remain largely overlooked. In parallel, there is a growing interest in studying cognitive syndromes in Vertebrates as the inter-individual level of analysis offers novel opportunities to explore the factors determining cognitive proficiency, the underling mechanisms and the associated fitness among other scientific applications. With this project, we first confirmed the consistency over time of individual cognitive performances, prerequisite for the existence of cognitive syndromes. Then, we evidenced correlation of performances within sensory modalities independently of the learning task but not between sensory modalities. We also observed cognitive specialization among tasks with bees showing higher level of flexibility in a reversal learning task while others were more skilled in configural processing (negative patterning task) suggesting the existence of cognitive modules relying on different neurobiological structures. Finally, we explored the potential factors that could impact variability in cognitive performances such as reward motivation, perceptual abilities or genetic diversity. Overall our results evidenced cognitive specialization within foragers which may have influential ecological impact and pave the way for further dissecting the neurobiological and developmental factors promoting key cognitive faculties in bees.

T12.3

Unraveling the neurophysiological underpinnings of visual identity recognition in a paper wasp

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Polistes fuscatus paper wasps possess individually distinctive color patterns on their faces, which can be used to recognize individuals on the nest. Behavioral assays demonstrate that individual recognition in this species is mediated by face-selective visual processing mechanisms. Using an extracellular 64-channel electrophysiological recording setup we sought to identify visually selective circuitry related to facial recognition. While simultaneously recording across the mushroom bodies, protocerebrum, and late optic processing neuropils, we presented visual stimuli including: frontal wasp views, side wasp views, silhouettes of wasps, other non-wasp images and canonical visual stimuli. A subset of neural responses in the mushroom bodies and protocerebrum are highly selective to frontal views of wasps. Critically, we have identified multiple wasp-selective neurons each from unique recording subjects and all possess similar physiological properties. Furthermore, these neurons are unresponsive to motion or other canonical visual stimuli. Incredibly, the responses of this population of wasp-selective neurons predicts the similarity between wasp facial patterns in multi-dimensional phenotype space. These neurons provide a key target cell population for further understanding the neural underpinnings of identity recognition in these special paper wasps. Our results in paper wasps echo the well-studied face-selective neurons that mediate visual individual recognition in primates, suggesting aspects of visual social recognition circuits have convergently evolved in primates and wasps.

T12.4

Olfactory learning and dopaminergic modulation in dipteran antennal lobes

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Olfactory learning in the wild is a far more complex exercise than choosing between two arms of a T-maze. Remembering the location of a ripe smelling apple in an orchard full of rotten fruit, manure, and leaves requires filtering of non-salient information. How do important odors get encoded into memory while background odors do not? Dopaminergic neurons in the vertebrate olfactory bulb and the insect antennal lobe assist in this task by modulating gain of olfactory signals projecting to higher order brain centers. Across Dipteran species, we observe different patterns of dopaminergic innervation to the antennal lobes with some expressing a diffuse pattern of dopaminergic neurons and others expressing concentrated innervation to certain olfactory glomeruli. After training in a classical conditioning paradigm, mosquitoes were able to learn to associate certain odors with a mechanical shock while other odors were not learned. The pattern of odors learned varied across species with different host preferences. What is the relationship between dopaminergic modulation and selective learning of odors? In the mosquito *Aedes aegypti* and the vinegar fly *Drosophila melanogaster*, we used 2-photon calcium imaging to observe the effects of dopamine modulation on odor encoding in glomeruli with different levels of innervation. I will discuss the potential relationship between differential dopaminergic innervation, odor salience, and olfactory learning in mosquitoes and flies. Changes in dopamine expression across olfactory glomeruli may be an effective adaptation for species-specific changes in learning behaviors.

T12.5

Innate cognition: Nest building as an example of tool use in an African cichlid

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Nest building has evolved in various invertebrate and vertebrate taxa. Depending on the ecological niche and the animal's cognitive abilities, building behavior can vary from being an invariant sequence of motor actions to a complex behavior involving search, appropriation and manipulation of specific materials. We are interested in the neuronal underpinnings of nest building behavior in the teleost fish *Lamprologus ocellatus* (LO), a cichlid species that is endemic to Lake Tanganyika. LO reside and exclusively breed in empty snail shells, which they embed vertically into the ground and cover with sand, leaving only the shell opening uncovered and accessible. To do so, LO performs a sequence of finely coordinated actions that can be reliably observed in the wild but also in an aquarium setting. We are analyzing how nest building is orchestrated in time and space, using computer vision and object detection algorithms to discern the kinematic structure and transition probabilities of individual behavioral motifs. To understand whether the behavior is governed by a fixed action pattern or a stimulus-response feedback loop, we are experimentally disrupting the interactions of the fish with the shell. We are also investigating shell object preferences by giving individuals a choice between 3D-printed shells with altered external or internal features. Finally, we are investigating which aspects of this behavior are innate by analyzing the building behavior of 'shell-naïve' LOs, i. e., individuals that were reared without physical contact to shells. The broader goal of this work is to establish a new vertebrate model for studying tool use in the context of a complex behavior with the prospect of discovering the underlying neuro-cognitive mechanisms.

T12.6

Bimodal sensory integration and learning in the vinegar fly *Drosophila melanogaster*

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Insects utilize a myriad of sensory signals to navigate natural environments. They show remarkable abilities to learn these cues and form memories associated with them. This learned information is essential to make crucial decisions at a later point in time. Conventional conditioning experiments have been used in the past to observe associative learning abilities of vinegar flies (*Drosophila melanogaster*) using individual sensory modalities. In our work, 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 an additional modality during training aids in better learning of visual and olfactory stimuli and in the retention of these memories. The results from these behavioural experiments provide evidence for the presence of neuronal substrates that can integrate sensory information and use that to form associations. Further physiological investigation in the higher brain regions such as the mushroom bodies and the lateral horn can reveal the identities of these neurons that are involved in multimodal information processing.

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POSTER COMMUNICATIONS

POSTER SESSION I
(Monday, 15:00–18:00)

SPATIAL ORIENTATION AND NAVIGATION I

A1 How the mushroom body and central complex contribute to visual homing in insects?

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Desert ants are excellent navigators, who use visual and other information to robustly forage and return to their nest. It is assumed that two parallel systems always run in the background while ants try to navigate: path integration (hypothesised to be the primary function of the central complex neuropil of the brain) and context-dependent visual place recognition (hypothesised to be part of the mushroom bodies function), both of which contribute in navigating to distant food sources or back to the nest location. It has been recently shown that the two systems eventually converge in the fan-shaped body of the central complex, suggesting that this is probably the primary centre for localisation and navigation in insects. Here, we use computer simulations of the natural environment and of desert ant sensors, along with anatomically constrained computational models of the mushroom body and central complex, to study how the visual information maps to behavioural output in the context of navigation. By using a theoretical approach, we show that decreasing the correlation amongst the connection patterns between visual projection neurons and Kenyon cells (input neurons) in the mushroom body, its performance for visual place recognition increases by at least 188%. Output to dopaminergic neurons' feedback connections enable self-reinforcement in the circuit, allowing increasing certainty for familiar views that come consecutively but not necessarily in the order experienced before. Finally, we hypothesise a model that samples the gradient of visual place recognition signals using the mushroom body and integrates them with path integration in the fan-shaped body of the central complex, producing steering commands for navigation.

A2 Dynamic properties of compass neurons in the bumblebee brain

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Bumblebees use the polarization pattern of the sky for orientation. Polarization information is processed in the sky-compass pathway and provides a reference for compass neurons in the central complex (CX). Tuning of such neurons has previously been characterized using polarized-light stimuli that rotate at a slow, constant velocity. Dynamical properties of such stimuli are far removed from those perceived by flying bumblebees. They typically perform rapid saccadic yaw turns, at angular velocities of up to 2000°/s, alternating with translational flight (Boedekker et al. 2015). To understand compass neurons' dynamic properties, we recorded intracellularly while stimulating with UV-light passed through a polarizer that was rotated according to head orientations recorded from flying bumblebees (Boedekker et al. 2015) or, as control, continuously at discrete velocities (30-1920°/s). CX-neurons showed consistent spiking during presentations of the naturalistic stimulus, suggesting that they reliably encode this stimulus. Stimulation at discrete velocities resulted in phase-locked responses up to 1920°/s. Phase-locking was even found when neurons did not fire at each stimulus rotation. For high velocities (≥240°/s) the firing phase shifted, representing response delays of the pathway, while at low velocities, the firing phase advanced. We explain this using a model that implements rebound effects after inhibition or excitation. Our results suggest that heading information cannot be extracted from individual CX-neurons, since their polarized-light tuning depends on stimulus velocity and history. Modelling of a population of neurons with individual polarization tuning showed that head orientation can be extracted from the population with an error dominated only by system delay.

A3 Natural switches in behavior rapidly modulate position by distance coding in hippocampal neurons

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In the real world, both humans and animals constantly switch between different behaviors. However, neuroscience research typically studies the brain while the animal is performing one behavior at a time – and little is known about how brain circuits switch between different natural behaviors. Navigation is a rich behavior that enables testing these behavioral switches: It requires the animal to know its own location within the environment, while also paying attention to incoming sudden events such as obstacles, predators or conspecifics. Here we set out to test how brief attentional switches to 'things out there' affect the representation of space in the hippocampus. We introduced pairs of bats to a 135-meter flight tunnel, alternating between flying alone and flying towards each other ('cross-over'). During cross-overs, bats had to be attentive to their partner to avoid collision. Indeed, they increased their echolocation-rate ~20 meters before the cross-over moment, indicating high attention in these events. We recorded the neural activity in hippocampal area CA1 and tracked the bats' positions using wireless-electrophysiology and custom tracking devices. We found that during cross-overs, many CA1 neurons encoded the distance to the other bat: The neurons switched from their place tuning to a conjunctive distance-by-place coding. These neuronal switches were very rapid, as fast as 100 ms, and were correlated with the bat's attention. Interestingly, the different place-fields of the same neuron often exhibited very different tuning to inter-bat distance. Overall, our results suggest that during dynamic natural behavior, neural circuits can rapidly switch between several computations, representing the relevant behavioral variables – to support behavioral flexibility.

A4 Under the real sky: compass neuron responses

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Many insects, including bees, desert ants, and flies use celestial cues for navigation. Locusts use the polarization pattern of the sky and the azimuth of the sun. Laboratory experiments have shown that polarization pattern and sun position are represented in the locust central complex (Zittrell et al. 2020, PNAS 117:25810). We compared responses of polarization-sensitive units to artificial stimuli and the natural sky. Extracellular multi-unit recordings with wire tetrodes from the locust brain were done in a hut on top of the Biology Building, which provided an unobstructed view of the sky. Responses to laboratory stimuli were recorded in the dark hut. First, the animal was illuminated from above and a polarizer was rotated by 360° above its head while the animal was stationary. Secondly, the animal was rotated by 360° while the polarizer was kept stationary. Afterwards, two hatches in the roof of the hut were opened. Polarization and spectral parameters of the sky were recorded with a camera and a spectrometer. Then, the animal stage was lifted above the roof and the animal was rotated by 360° under the natural sky. Recordings lasted for one to three hours allowing for one to three repeats of laboratory and natural sky stimulation. Responses to the artificial stimuli had axial preference angles, which were independent of whether the animal or the polarizer were rotated. In all but one unit, responses to the natural sky changed to a circular representation. The preference angles diverged by about 90° from the axial representations. This suggests azimuthal preference relative to solar position and/or matched filter coding for sky polarization. Thus, we currently test how representations change when either the sun is obscured or sky polarization is eliminated.

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A5 Neuronal control of turning behavior in freely flying flies

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The flight of *Drosophila melanogaster* is characterized by two distinct phases: segments of straight flight, which are interspersed by sharp turns called saccades, in which the fly performs a fast (40-60 ms) banked turn, either spontaneously or to avoid collisions. Previous research into the control of these turns has focused on descending neurons - a group of a few hundred pairs of neurons that integrate information from the brain of the fly and transform them into motor commands sent down the ventral nerve cord. This research revealed a descending neuron that may directly control saccades. However, it is still unclear whether this behavior is truly controlled by a single neuron, or if it requires the concurrent activity of multiple descending neurons. Additionally, this research has been limited by focusing on tethered flight experiments, a condition qualitatively different than free flight. In order to overcome this limitation, and to study the exact nature of descending neuronal control during flight in Drosophila, we have developed and constructed a free-flight arena, which allows tracking naturally behaving flies while also enabling optogenetic activation of selected neurons using stochastic expression of the CsChrimson light-activatable cation channel. Using this setup, we are able to show that activation of a specific descending neuron is enough to elicit a turn in free-flight, which bears a striking resemblance to natural saccades. Additionally, we have found that flies perform a short counter-turn following the initial, optogenetically-induced turn. Using this setup, we hope to further elucidate the exact mechanism by which descending neurons control flight behavior in fruit flies.

A6 Bumblebees dash through an artificial forest by combining different guiding mechanisms

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Following habitual routes to commute between locations is an everyday task and a widespread navigational strategy used by many animals. Bumblebees, Bombus terrestris, as well as other foraging insects, are excellent navigators that can travel long distances to look for food sources. They were observed to travel along routes and optimize routes between multiple feeding locations. While foraging, obstacles require the bees to deviate from their path in order to avoid collisions. However, it remains unknown how bees establish these routes and how experience affects the flight paths of bees in clutter. On the one hand, route following might be visually guided, and bees establish routes by matching the current view with familiar visual memories along the previous journeys. On the other hand, route following might be a by-product of bees aiming for their nest (e.g.guided by path integration) and spontaneous reaction to avoid collisions. By recording flights of bees in an environment with multiple obstacles (i.e. clutter), we show that bees quickly learn to efficiently cross the clutter and their flight paths converge to distinct routes they follow to return to their hive. Bees display a behavior that could be explained by an interaction of different mechanisms, such as following the most familiar scenery, avoiding an obstacle, while aiming for their nest, along their routes. We studied the dynamical orchestration of these mechanisms by comparing model responses to the flight path, by using a novel causality detection method (convergent cross-mapping). Our findings shed light on the route following mechanisms that may drive bees at different stages of their foraging life.

A7 Homing of bees in cluttered environments

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Along a foraging journey, flying insects, such as bees, are guided by the current visual sceneries. Learning relevant aspects of these views is critical as they are necessary to pinpoint the nest on later returns. During such learning flights when exiting the nest for the first time, bees gain flight altitude and increase their distance and height to the nest. They exceed the height of objects surrounding the nest, such as flowers, grass, or bushes, and they experience views entirely different from those at much lower altitudes near the nest. In a cluttered environment like a forest or a meadow, the views around the nest may be ambiguous to the similarly-looking objects and numerous occlusions whereas the views above the clutter are not due to a unique pattern formed by the object's arrangement on the floor. The question arises of how bees return to their nest in clutter and how they use views from varying altitudes. We challenged bumblebees to pinpoint their nest entrance in a lab setting surrounded by randomly placed objects. To study their use of altitude to home, we restrained their flight altitude, during learning, homing, or both. Even bees that could fly above the clutter while learning, meandered through the clutter to their visually indicated nest location. The bees used multiple routes to enter the clutter between the objects, indicating that the cluttered objects did not constrain the bees to enter only at a certain position. A comparison with image difference-based homing models could not account for the homing performance of the bees within the clutter. Our experiments could show that bees can pinpoint the nest within a cluttered environment without using views from above. These results suggest that bees can return home without crossing the path of their learning flights.

A8 Characteristics of *Cataglyphis*' magnetic compass

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Greek *Cataglyphis nodus* ants use the geomagnetic field to align their gaze directions to the nest entrance during initial learning-walk pirouettes. Pirouettes by so-called novices are characterized by frequent stopping phases. During the longest stopping phase, ants gaze into the direction of the nest entrance. This behavior offers an ideal readout to understand the characteristics of *Cataglyphis'* magnetic compass in more detail. We used different coil systems, i.e. an electromagnetic spiral, differently sized rectangular coils, a Helmholtz coil, and a 3D Helmholtz coil, to alter the geomagnetic field in the natural habitat both around the nest entrance and at a feeder. The different setups allowed to alter the magnetic field in various manners, for example by eliminating, strengthening, and rotating only the horizontal or vertical component or the complete magnetic field. Elimination induced drastic behavioral changes whereas strengthening did not change the ants' behavior. Only novices at the beginning of their outdoor lives adapt their gaze directions relative to the magnetic field. We hypothesize that *Cataglyphis* novices use a polarity sensitive magnetic compass during initial learning walks. In contrast, experienced foragers are magnetosensitive, but do not rely on the magnetic field as a reference system to align their gaze directions towards the nest or the feeder.

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A9 Quantitative description of a flight trajectory by automated segmentation: example of moths in wind tunnel

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Animals move through complex trajectories, but these are classically described with very simple observations: latency before reaching a point, proportion of animals meeting a criterion... Modern trajectory recordings can provide coordinates of animal in two or three dimensions at all times; yet this is difficult to exploit directly, especially for comparing animals subjected to different experimental conditions. Here, we present an automated method to describe trajectories by dividing them into segments. The segmentation algorithm starts by considering the trajectory is formed by only one segment, then makes two segments at the level of the trajectory point most remote from this initial segment; this procedure is reiterated for each segment until all the points are below a threshold chosen beforehand. The advantage of this method is to produce units of analysis (segments) useful for comparing the trajectories between different experimental groups. To do this, it is possible to take advantage of modern statistical methods for repeated measures (mixed models), since there are several segments per trajectory. We illustrate this with male *Agrotis ipsilon* moths exposed to sex pheromone in a wind tunnel, in the presence of different olfactory environments. These had no effect on the parameters studied, whereas the presence of pheromone increased turn angles (angles between segments) and decreased speed. Moreover, the magnitude of turn angles predicts the probability of finding the pheromone source. Finally, aligning trajectories indicates turning angles increased just before approaching the target in the presence of pheromone. This analysis of the trajectories was only possible thanks to segmentation, illustrating the interest of this method.

A10 A hypothesised network for communicating vector information in the honeybee waggle dance

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In the honeybee waggle dance, a forager communicates the location of valuable resources to nestmates inside the hive via a mixture of body motion, mechanical and chemical cues. The location information used by the dancer is postulated to have the internal form of a flight vector which has been acquired via path integration. Similarly, nestmates that follow the dance are assumed to acquire the location in the form of a flight vector that they can follow to the resource. This suggests performance of the dance and assimilation of the information by nestmates could have a common neural mechanism. The central complex is a well-mapped neuropil in the insect brain important for oriented behavioural responses. In this work, we use a computational approach to explore how a network for path integration modelled on the central complex might account for the needs of both dancers and nestmates to respectively represent, communicate and assimilate the location of a resource. We investigate how this network can support the necessary intrinsic computation and propose additions to the network which reveal aspects of the natural waggle dance behaviour. We also consider how nestmates utilise sound and antennal inputs to obtain the information.

A11 Decoding the ultimate compass: A neural substrate for multimodal cue integration in insect orientation

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Travelling in a straight line is more taxing than one would think. Without an allothetic orientation cue, any system will eventually fail due to motor noise. Ball-rolling dung beetles are capable of very robust straight-line orientation behavoiur using a variety of different orientation cues, thought to be stored in an orientation 'snapshot'. Recent work suggests that these cues are likely integrated using a form of vector summation; angular cues are represented as vectors with matching polar angle, the magnitudes then determine the influence in the integration. Here we present a neural model of the insect head-direction circuit. Our results illustrate how this network could encode a vector sum for orientation cue integration. Further we show that, with a relatively simple learning rule/procedure, the network can learn the spatial arrangement of cues in the environment. This learning procedure may also store information about the usefulness of a particular cue, providing an emergent explanation for cue weighting in the integration process.

A12 Fragmented replay of very large environments in the hippocampus of bats

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↑UP

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In sleeping or immobile rodents, ensembles of hippocampal place-cells exhibit sequential reactivations of previously-experienced trajectories (termed 'replay'). Such neuronal sequences are thought to be important for memory consolidation. Here we searched for replay sequences in the hippocampus of bats flying in a 200-meter long tunnel. We asked: Are there ultra-long replay sequences in bat hippocampus, which cover the entire large naturalistic environment? To investigate this, we decoded neuronal activity when bats were stationary – during rest-times between flight epochs in the 200-meter long tunnel, and during subsequent sleep. The decoding analysis revealed many replay sequences. Surprisingly, these sequences depicted trajectories that covered relatively small pieces of the environment, between a few meters and ~20 meters – i.e. less than 10% of the environment size – in striking contrast to replay sequences in rodents in small setups, which typically cover the entire environment. These sequences were time-compressed, with a compression-ratio similar to rodents. These findings provide the first demonstration of hippocampal sequential replay in a non-rodent species. Our findings may have important implications for understanding hippocampal replay in mammals. Specifically, the fragmented replay of information in this large environment might reflect: (i) Communication between hippocampus and neocortex – where the short replay fragments resemble the small information-packets used for transmitting large messages in artificial communication systems. (ii) Training of the neocortical network by the hippocampal network, using snippets of long experiences – as seen in training protocols of deep neural networks.

13 An intrinsic oscillator underlies visual navigation in ants

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Navigation implies a constant balance between exploration – to gather information – and exploitation – to use this information to reach one's goal. However, how this trade-off is achieved in navigating animals is unclear. Here we recorded the paths of two phylogenetically distant visually navigating ant species (*Myrmecia croslandi* and *Iridomyrmex purpureus*) using a trackball-treadmill directly in their habitat. We show that both species continuously produce regular lateral oscillations with bursts of forward movement when facing the general direction of travel, providing a remarkable trade-off between visual exploration across directions and movement areas. This dynamical signature is conserved across navigational contexts but requires certain visual cues to be fully expressed. Rotational feedback regulates the extent of turns, but is not required to produce them, indicating that oscillations are generated intrinsically. Learnt visual information modulates the oscillation's amplitudes to fit the task at hand in a continuous manner: an unfamiliar panorama enhances the amplitude of oscillations in both naïve and experienced ants, favouring visual exploration; while a learnt familiar panorama reduces them, favouring exploitation through. The observed dynamical signature readily emerges from a simple neural-circuit model of the insect's conserved pre-motor area known as the lateral accessory lobe, endorsing oscillations as a core, ancestral way of moving in insects. We discuss the importance and evolution of self-generated behaviours and how such an oscillator has been exapted to various modalities, behaviours and way of moving.

A14 Specification of a goal direction by local neurons in the *Drosophila* fan-shaped body

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During navigation, animals must rapidly and flexibly alter their goals, and maintain them in the presence of noise or conflicting goal information. How such goals are represented in the brain is unclear. A ubiquitous form of goal-directed navigation is flow-directed olfactory navigation, in which animals orient and navigate upwind upon encountering an attractive odor. Work from our laboratory has identified hΔC local neurons in the fan-shaped body (FB) as a possible locus of goal information for olfactory navigation. hΔC neurons receive input both from

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wind-tuned columnar FB inputs, and from odor-tuned tangential FB inputs that drive upwind navigation. Imaging experiments reveal that hΔC neurons encode an odor-gated wind direction-tuned signal. Here we show that sparse activation of hΔC neurons drives reorientation and walking in a persistent reproducible direction, consistent with their encoding a goal heading. In contrast, broad activation of hΔC neurons drives unstable re-orientation. Based on motifs from the *Drosophila* connectome, we develop a computational model showing how hΔC neurons can promote upwind navigation, a persistent reproducible walking direction, or unstable re-orientation, depending on their activity pattern. Our model provides a mechanism for rapid and flexible, yet robust specification of a goal heading, and suggests testable hypotheses for the function of additional FB neuron types.

A15 Object × position coding in the entorhinal cortex of flying bats

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Successful navigation requires knowledge of the locations of specific objects, such as landmarks and goals. However, it is unclear where in the brain this information is integrated into the cognitive map of space So far, only an object-invariant signal was discovered, carrying general information regarding all objects at all locations (carried by so-called 'object-vector cells' in the superficial layers of the medial entorhinal cortex (MEC)). It is thus unknown whether and where this general object-related signal converges with a signal for a specific location (as carried by place cells in the hippocampus) – and whether these two variables are encoded conjunctively. We hypothesized that if indeed the brain represents object × position in a conjunctive manner, such encoding will be found at the "end" of the MEC-hippocampal-MEC loop – namely, the deep layers of MEC – where all-object information from MEC converges with location-specific information from hippocampal place-cells, and is then sent to the neocortex. Here we recorded from MEC of flying bats as they foraged for food in a large flight-room where 6–11 identical rest-objects were placed at various heights and locations. We found that a substantial fraction of cells in the deep layers of MEC (but not superficial layers) fired at the vicinity of specific rest-objects at specific locations. These cells fired near the rest-object when the bat flew from or to the object, but not when it flew through the same location without object-engagement – thus encoding object × position. Our results suggest a broader prevalence than currently thought for conjunctive coding of navigational variables – including the encoding of objects, which are crucially important for navigation.

A16 Underlying mechanisms at play during learning flights

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Ants and bees hatch in their dark hive, visually isolated from the surrounding. Some become foragers at one stage of their life, travelling back and forth between food sources and their hive. On their first excursions outside the hive, the individual, be it an ant, a bee, or a wasp, performs peculiar manoeuvres. These manoeuvres compose a so-called learning walk or flight during which the visual surrounding of the environment is learned and navigational mechanisms (part of a navigational toolkit) are calibrated. Thus, before engaging in the quest for food, the individual appropriately manoeuvres to enable the return to an often inconspicuous hive hidden under the ground or within the vegetation. The individual can learn and guide their return with a plethora of olfactory, tactile, or visual cues. For example, bees can use the objects' spatial arrangement, their colours, and the lines formed between the sky and structure on the ground to pinpoint their nest entrance. Thanks to the joint effort of modelling and experiments, the underlying mechanisms at play guiding the individual to their hive again gained clarity. However, most of the modelling efforts focus on the navigation of ants constrained by a mode of locomotion (walking on a surface) different to bees. They also contain several dissimilarities with the shape of learning walks and flights. Here, I will review the findings in ants, bees, wasps, and the suggested models to discuss the potential underlying mechanisms at play during learning flights in a variety of landscapes.

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A17 Hippocampal encoding of egocentric 3D target location in the echolocating big brown bat, *Eptesicus fuscus*

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Echolocating bats emit sonar signals and process auditory information carried by returning echoes to represent the spatial layout of objects in their surroundings. The bat collects echo information from the environment to carry out a variety of tasks, such as foraging, obstacle avoidance, navigation, and social communication. A growing body of research details spatial representation in bat hippocampus, but studies have yet to investigate hippocampal encoding of 3D sonar target location. To bridge this gap, we are exploring hippocampal encoding of egocentric target distance in the FM bat, Eptesicus fuscus, as it performs a sonar target-tracking task. The echolocating bat actively controls the features of its sonar calls in response to information it collects from objects in the environment, and therefore, the bat's adaptive echolocation behavior provides a direct metric of its spatial attention to objects. In our experiments, bats were first trained to perch on a platform and track a target (cluster of tethered mealworms) that moved towards and away from the bat at a starting distance of 3m. The bat's echolocation calls were acquired with an array of microphones and digitized, and its head aim was tracked with a high-speed video system. Multichannel neural recordings were taken from hippocampal CA1 in freely echolocating bats and synchronized with audio and video data. Spikes were sorted offline, and response areas of single neurons were quantified. Our preliminary data show that a population of hippocampal CA1 neurons encode the 3D egocentric location of targets and spatial tuning is modulated by sonar-guided attention.

A18 Visual pursuit behavior in mice maintains the pursued prey on the retinal region with least optic flow

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Mice have a large visual field that is constantly stabilized by vestibular ocular reflex (VOR) driven eye rotations that counter head-rotations. While maintaining their extensive visual coverage is advantageous for predator detection, mice also track and capture prey using vision. However, in the freely moving animal quantifying object location in the field of view is challenging. Here, we developed a method to digitally reconstruct and quantify the visual scene of freely moving mice performing a visually based prey capture task. By isolating the visual sense and combining a mouse eye optic model with the head and eye rotations, the detailed reconstruction of the digital environment and retinal features were projected onto the corneal surface for and updated throughout the behavior. By quantifying the spatial location of objects in the visual scene and their motion throughout the behavior, we show that the prey image falls within a small area of the VOR-stabilized visual field. This functional focus coincides with the region of minimal optic flow within the visual field and consequently area of minimal motion-induced image-blur, as during pursuit mice ran directly toward the prey. The functional focus lies in the upper-temporal part of the retina and coincides with the reported high density-region of Alpha-ON sustained retinal ganglion cells.

A19 How has artificial selection changed olfactory bulb anatomy in the homing pigeon (*Columba livia*)?

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The pigeon (*Columba livia*) has been domesticated for thousands of years and selected for a range of specific behaviors, plumages, and body sizes. Although all pigeon breeds are the product of artificial selection, one of the most extreme outcomes of artificial selection is the homing pigeon's incredible ability to navigate. Homing pigeons can navigate to a home loft over thousands of kilometers of unfamiliar terrain. Although spatial memory and multiple sensory cues are used to successfully home, homing pigeons rely heavily on olfactory cues to orient in the direction of their home loft and maintain the correct direction for their entire flight. Here, we tested whether selection for homing has altered olfactory bulb (OB) anatomy in the homing pigeon compared with other pigeons. Using unbiased stereology, we quantified OB size as well as the number and size of mitral cells in the OB among homing, feral, show, and active pigeon breeds. Homing pigeons had the largest olfactory bulbs (+28-72%) and had more mitral cells (+13-50%) than any other breed examined. Relative to the cerebral hemispheres, homing pigeons had larger OB's than almost all other breeds, but not proportionately more mitral cells. Conversely, homing pigeon mitral cells tended to be smaller than those of other breeds. We conclude that selection for homing has driven an expansion of the olfactory bulbs in homing pigeons without altering mitral cell density or size. How this translates to differences in olfactory abilities remains to be determined, but we predict that homing pigeons have better sensitivity and acuity than other pigeons in order to improve the precision and accuracy of olfactory-guided homing.

A20 Selection for homing has driven an increase in hippocampal neuron numbers in the homing pigeon

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Domestication is the process by which animals are artificially selected over generations for a variety of desired morphological and/or behavioural traits. One of the most famous examples of artificial selection for behaviour is the homing pigeon. Homing pigeons can locate their home loft from a novel start point over thousands of kilometres while they are 'homing', a spatial task dependent on the hippocampal formation (HF). However, despite breed differences in spatial-cognitive performance, it remains unclear if homing pigeons differ in HF anatomy from other breeds. Here, we test if homing pigeons have larger HF volumes with more neurons than other pigeons, including ferals, show, and sporting breeds. Using unbiased stereological methods, we measured the volumes of the HF and septum as well as number of neurons within both regions. Homing pigeons have larger HF volumes than other breeds (10-24%), but there was considerable overlap with almost every other breed examined. Similarly, when scaled for telencephalon size, homing pigeons had proportionally larger HF volumes than some, but not all, breeds. The difference in neuron numbers was more substantial; homing pigeons have more neurons and higher HF neuronal density than other breeds, with the biggest contrast between homing and feral pigeons (>200%). Homing pigeons did not, however, differ in size or number of neurons in the septum, indicating that the differences were specific to the HF. We conclude that selection for homing has resulted in increases in the number of HF neurons in homing pigeons, providing the first evidence of artificial selection for a cognitive task driving a change in neuron number and density.

A21 Do bumblebees rely on magnetoreception to perform a spatial memory task in the absence of visual and olfactory cues?

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Many living organisms, from bacteria to birds, are known to perceive the Earth magnetic field (or geomagnetic field, GMF), and some use their magnetoreceptive ability for navigation. Bees can sense magnetic fields, but the function of magnetoreception remains unclear. This knowledge gap needs addressing, especially in a global picture where insects are declining at unprecedented rates, and there is considerable concern for the possible impacts of electromagnetic radiation generated by human infrastructures on the ability of pollinators to undertake basic tasks that require navigation, such as foraging. In a series of experiments, we have investigated magnetoreceptive behaviour in the bumblebee *Bombus terrestris* in an indoor setup. The bees were trained to a feeder in an arena where the only cue available was the GMF; then the performance of the control group vs. two treated groups was compared. The treatments consisted of a +/-90 degrees shift of magnetic North. Initial findings show that applying a magnetic manipulation that mimics a shift in the GMF direction can induce a change in behaviour. In particular, we observed that not only did treated bumblebees fail to search for the feeder in the training direction, but some bees also did not orient in any specific direction at all. A second series of experiments aims to clarify and quantify the nature of the effect by training the bees in a changed magnetic field and then test their navigational preference with the field deactivated.

A22 Odour disambiguates visual conflicts for homing bumblebees

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Most social insects such as ants, honeybees, and bumblebees are excellent navigators. For example, bees can learn multiple locations in their environment, like flower patches and their nest. While navigating from one location to another, bees mainly rely on vision. In general, the visual environment of a bee such as a meadow or a garden is reliable; yet it can be prone to rapid changes such as moving shadows or the displacement of an object in the scenery. Therefore, bees may use multimodal guidance to ensure their return to their goal location. Alternatively to vision, they might use another well-developed sense: olfaction. Olfaction is used for a wide range of functions such as communication, mate recognition or designation of depleted flowers. However, little attention has been paid so far to a role of odour in navigational tasks. In addition, potential odour cues are often not considered in common experimental designs. Therefore, I tested whether bumblebees (*Bombus terrestris*) use scent marks deposited by them around their nest during homing behaviour in visually ambiguous situations. Bees were trained to return to their inconspicuous nest hole in an indoor flight arena by using a specially designed visual scenery and scent marks around their nest entrance. Upon a forager's return, the visual scenery was altered so that it indicated two possible nest locations, one of which was additionally equipped with the collected odour from the original nest hole. I could show that bumblebees actively shape their search behaviour at the potential nest locations and can use natural scent marks to disambiguate environmental conflicts under visual uncertainty

A23 Learning and memory in desert ants: insights from fine-scale reconstructions of the entire foraging history of individual foragers

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Multiple ant species navigate between their nest and feeding sites using visually-guided routes but the learning needs and underlying control strategies remain unknown. In this work, we tracked desert ants from the first time they left the nest until they established a regular feeding site ~8m from the nest, before probing their visual homing performance using displacement trials. Using our path reconstruction software we analyse these paths in unprecedented detail and assess the impact of motivation and environment on behaviour. We find that two trips (with only one completed) are sufficient for learning visually guided routes paths and that learning walks are not essential to success. Moreover, we report differences in the learning of outward and inward routes which we map to distinct goals and environmental interactions. Finally, we report adaptive oscillatory paths in different environmental contexts that drive distinct search and route alignment behaviours.

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MOTOR SYSTEMS, SENSORIMOTOR INTEGRATION, AND BEHAVIOR I

B1 Stride-coupled modulations in *Drosophila* visual neurons guide rapid and flexible walking course control

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We must rapidly monitor and adjust our body movements to achieve behavioral goals. Movement adjustments must be flexible and tuned based on both the behavioral goals and the ongoing state of the body. How the nervous system achieves such rapid and flexible motor coordination remains poorly understood. Here, we leveraged a compact and well-defined walking course control circuit in the *Drosophila* visual system to investigate how sub-second neural activity dynamics reciprocally interact with fine leg movement parameters. We performed a whole-cell patch-clamp recording from a class of optic-flow sensitive cells, HS cells, while a fly walked spontaneously on a spherical treadmill ball. We found that HS cells receive extraretinal periodic modulations coupled to leg stride cycles. The stride-coupled modulation was dependent on the activity of leg mechanosensory neurons and a pair of newly identified ascending neurons. The modulation contributed to walking course control on multi-timescales: On a stride timescale, the periodic modulation sets a time window within which HS-cell activity is rapidly tuned contingent on a course drift. Brief HS-cell depolarization promoted corrective steering via the ipsilateral front leg's stance extension. On a longer timescale, the signal is gradually integrated over several strides to represent a steady forward speed. This speed context representation recruits the circuit for course control as needed. Therefore, the stride-coupled modulations are tailored for rapid and flexible course control. The discovery of such a fast walking-related representation in the insect brain opens up the opportunity to study single cell-level mechanisms for stride-based computations such as path integration for navigation and motor control.

B2 Synaptic drive from central pattern generating networks to leg motor neurons differs between leg joints in an insect leg muscle control system

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Rhythmic motor behaviors, such as walking, result from the interaction of neural circuits that can produce a rhythmic motor output, called central pattern generators (CPGs), with feedback signals from leg sensory organs monitoring movement and force. In the stick insect individual CPGs exist, which provide alternating rhythmic synaptic drive to the antagonistic motor neurons (MN) innervating the muscles of each of the three main leg joints, i.e. the thoraco-coxal (ThC-), the coxa-trochanter (CTr-) and the femur-tibia (FTi-) joint. FTi-MNs receive alternating rhythmic inhibitory synaptic drive from the respective FTi-CPG (Büschges, 1998). We hypothesized that rhythm generation in the other motor neurons, i.e. those controlling ThC- and CTr-joints, is based on the same mechanism. To test this, we pharmacologically induced rhythmic activity in MNs of the deafferented mesothoracic ganglion (Büschges et al. 1995) and recorded intracellularly from their neuropilar processes. Synaptic drive was analyzed by measuring input resistance and by artificially altering the membrane potential during ongoing rhythmic activity. We present data concerning the synaptic drive provided by the CPGs of the ThC- and the CTr-joints to the respective MNs. We show that the synaptic drive underlying rhythmic activity in MNs is joint-specific. Similar to FTi-MNs, alternating activity in ThC-joint MNs is based on alternating inhibitory synaptic drive to the two antagonistic MN pools. In contrast, rhythmic activity in depressor MNs of the CTr-joint was found to be generated by phasic excitatory synaptic input.

B3 Peripheral modulation of cardiac contractions in the American lobster, *Homarus americanus*, by the peptide myosuppressin is mediated by effects on the cardiac muscle itself

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Central pattern generators (CPGs) are networks of neurons that produce patterned motor outputs and drive behaviors such as swimming, breathing, and walking. Modulation of CPGs, largely due to peptides, creates variations in motor patterns that result in behavioral flexibility. Neuromodulators also have the capacity to alter muscle dynamics peripherally via the neuromuscular junction (NMJ) and the muscle itself. The cardiac neuromuscular system of the American lobster is driven by the cardiac ganglion (CG), which is a CPG comprised of nine neurons and is a model for peptide modulation. Myosuppressin is an endogenous neuropeptide that decreases contraction frequency and increases contraction amplitude by modulating both the CG and the periphery. Myosuppressin increases the duration of action potential bursts and decreases cycle frequency in the isolated CG. Peripherally, it increases contraction amplitude through a previously unknown mechanism. Here, we investigated one important remaining question: does myosuppressin exert its peripheral effects on the cardiac muscle, the NMJ, or both? To measure effects at the NMJ, excitatory junction potentials (EJPs) were recorded using a microelectrode inserted into a single muscle fiber; after removing the CG, we stimulated the motor nerve and superfused myosuppressin over the preparation. Myosuppressin did not modulate the amplitude of EJPs. To determine whether myosuppressin directly alters contraction of the cardiac muscle, the CG was removed, and muscle contractions were stimulated with L- glutamate while superfusing myosuppressin. Myosuppressin increased glutamate-evoked contraction amplitude in the isolated muscle, suggesting that myosuppressin exerts its peripheral effects directly on the cardiac muscle.

B4 Modulation of feedback pathways in the cardiac neuromuscular system of the American lobster, *Homarus americanus*

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In the American lobster, Homarus americanus, heartbeat is controlled directly by the nervous system through a pattern generating network known as the cardiac ganglion (CG). The lobster CG is composed of four small interneurons that stimulate five larger motor neurons, which in turn generate repeated bursts of action potentials that stimulate contraction of the cardiac muscle. Strength of cardiac contraction is dependent on burst frequency, duration, and patterning of impulses in the CG. The cardiac neuromuscular system is modulated by both neuronal and hormonal inputs, as well as by multiple feedback systems to ensure heartbeat flexibility and stability. By isolating the CG and surrounding muscle, we have characterized the complex yet generally excitatory response to cardiac muscle stretch, which is mediated by stretch-sensitive dendrites that extend from each of the nine neurons into cardiac muscle. We know from previous research that the cardiac neuromuscular system as a whole is heavily modulated by neuropeptides, which can act at multiple sites, including the CG itself and the periphery (neuromuscular junction and/or cardiac muscle). We determined that two neuropeptides, GYSNRNYLRFamide (GYS) and SGRNFLRFamide (SGRN), generally suppress the excitatory stretch response. In the intact heart, a second feedback pathway, mediated by nitric oxide (released by the cardiac muscle during contraction) opposes the stretch pathway by decreasing burst frequency and cardiac contraction amplitude. This raises the question of whether there are direct interactions between the two feedback pathways. We are thus currently asking whether NO, like the neuropeptides, modulates the stretch feedback pathway.

B5 Functional coupling of the mesencephalic locomotor region and V2a reticulospinal neurons driving forward locomotion

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Throughout vertebrate species, locomotion relies on high brain centres converging onto the mesencephalic locomotor region (MLR). The nature of the RSNs the MLR recruits to initiate and modulate forward locomotion remains obscure due to the heterogeneity and difficulty to access these systems. Here, we leverage the optically and genetically accessible larval zebrafish to investigate the recruitment of a genetically-defined population of RSNs conserved across vertebrates, referred to as V2a RSNs, during MLR-induced forward swimming. We identified for the first time, functionally and anatomically, the MLR in larval zebrafish. MLR stimulation reliably controlled the duration and locomotor frequency of forward swimming bouts. Using calcium imaging in combination with MLR stimulation we show that 20% of the medial V2a RSNs in the medulla were recruited during MLR-induced forward swims. Although a subset of these neurons maintained their activity throughout the duration of the swim, the kinematic of the calcium responses differed. Using multiple linear regression to model activity with motor regressors, we found that a cluster of V2a RSNs in the caudal medulla encoded locomotor frequency and number of oscillations, while another cluster in the rostral medulla encoded locomotor frequency and the increase in the movement amplitude. Our study reveals that the MLR specifically recruits a previously unappreciated population of V2a RSNs to control forward locomotion. The description of the MLR in larval zebrafish represents a breakthrough for future investigation of the supraspinal motor centres as virtually each and all RSNs can be identified, monitored and manipulated in this transparent genetic model organism.

B6 Recurrent inhibition in motor control

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The execution of rhythmic motor behaviors requires multiple control mechanisms to adjust the behavioral output, narrowing down the degrees of freedom of a system that involves many units. Leeches crawl on solid surfaces through a succession of elongation and contraction body waves, anchored on the posterior and anterior suckers. Each segmental ganglion contains all the neurons required to produce this rhythmic motor pattern, and dopamine evokes fictive crawling (*crawling*) in isolated midbody ganglia. The pair of premotor NS (nonspiking) neurons, similar to vertebrate Renshaw cells, are connected to motoneurons through a central network that provides recurrent inhibitory signals onto the motoneurons. We aim at understanding the role of NS in the context of *crawling*. During *crawling* NS neurons receive inhibitory signals, tuned to its contraction phase, monitored through the DE-3 motoneuron. The results suggest that the inhibitory signals in NS are delivered by the rhythmogenic circuit that controls the motoneuron output. Thus, excitatory signals onto DE-3 are correlated with inhibitory signals in NS that, in turn, could restrict the motoneuron activity. Extracellular recordings combined with spike sorting analysis allowed the simultaneous study of multiple motoneurons in the course of *crawling*. An NS manipulation that transiently removes the recurrent inhibitory pathway enhanced the firing frequency of motoneurons firing during the contraction phase, including DE-3, and expanded the duty cycle. At present we study the effect of NS on the phase relationship of motoneurons during *crawling*.

B7 Moving anchors: dynamics of ground reaction forces in freely behaving *Drosophila melanogaster* larvae revealed by deformable optical resonators

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Soft-bodied animals move over terrain through intricate interactions with substrates. Direct measurement of substrate interactions in small soft-bodied animals has proven challenging due to limitations in methods for measuring ground reaction forces with high spatiotemporal resolution. Here, we present a novel approach using Elastic Resonator Interference Stress Microscopy (ERISM), enabling visualisation and calculation of ground reaction forces during substrate interaction in freely behaving *Drosophila* larvae with micrometre scale spatial resolution and millisecond temporal resolution using optical cavities. Combined with detailed kinematic tracking of substrate-interfacing cuticular features, we uncovered a dynamic pattern of substrate interaction by pro-leg like cuticular protrusions. These 'protopodia' are selectively articulated during different phases of larval behaviour and provide anchoring for movement over surfaces beyond what is afforded by mucoid adhesion. We found that the forces produced by individual protopodia are in the 1-8 µN range. We also measured the dynamics of substrate interaction within protopodia and saw that the structures transiently articulate into shapes optimal for anchoring the larval body for propulsion and cantilevering. Overall, this work presents a first in-depth insight into the biomechanics underlying substrate interactions in a genetically tractable soft-bodied arthropod and provides a framework for further use of ERISM in animal biomechanics research.

B8 Sensorimotor apparatus underlying compensatory head-movements in hawkmoths

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During complex aerial maneuvers, insects achieve control by integrating feedback from multiple sensory modalities and generating coordinated responses that enable them to stay on their intended trajectories. These flight-related responses include generation of compensatory head motion, which enable insects to stabilize their gaze. Such head movements depend on a complex neck motor apparatus which ensures smooth neck motion in response to a combination of optic flow on the retina and vestibular feedback from mechanosensory modalities. In flies, the mechanosensory feedback is derived from halteres that detect angular rotations of the body during flight as well as prosternal organs which detect head orientation relative to body. Whereas flies strongly rely on halteres for mechanosensory feedback on body rotations, most insects lack halteres. This leaves open the question of how non-Dipteran insects derive mechanosensory feedback for gaze stabilization in flight. In a recent study on hawkmoths, we showed that in addition to visual feedback, the neck muscles also respond to antennal mechanosensory feedback from the Johnston's organs. Do prosternal organs, which are not described in hawkmoths, also play a role in head stabilization? To address this, we conducted a detailed characterization of the neck sensorimotor apparatus in the Oleander hawkmoth *Daphnis nerii* which contains many findings. First, we describe a field of neck mechanosensory bristles for the first time, similar to the prosternal organ in flies. Second, we conducted a detailed X-ray micro tomography characterization of the 3-D musculoskeletal architecture of the neck motor apparatus. Third, fluorescent dye labelling in the various neck muscles revealed the neural architecture of neck motor neurons in the brain.

B9 Neural control of inter-limb coordination in an amphibious fish – the mudskipper

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Land transition was a crucial step towards the evolution of terrestrial vertebrate life. Many aquatic vertebrates use undulatory movements along the axial body musculature for locomotion, with their pectoral and pelvic appendages mainly being used for posture control and steering. In terrestrial habitats, many animals use limbs for locomotion. To lift their body above the substrate, they need to produce forces in multiple directions to effectively move forward. This requires not only more complex pectoral and pelvic appendages (i.e. fore- and hindlimbs) that provide higher degrees of freedom for multidimensional limb-control – but also more complex spinal motor control networks (CPGs) that are adapted to this new functionality. We aim to understand the adaptations these circuits needed to undergo, in order to enable the coordinated movement of pectoral and pelvic fins. Such coordinated movement was necessary to allow stem tetrapod species their first steps on land. Mudskippers provide an excellent model to investigate adaptations within and between locomotor networks that enable land transition. They use the "ancient", fish-like undulatory locomotor program to swim using their tail. In addition, they use a motor program comprised of well-coordinated movements of the pectoral and pelvic fins to produce "crutching" terrestrial locomotion. We use backfills of axial, pectoral and pelvic nerves,

immunohistochemical staining and in-vivo locomotion tracking to unveil the anatomy, histochemistry and interplay of locomotor CPGs that coordinate swimming and walking in mudskippers. In-vitro electrophysiological studies are planned to further decipher the interconnectivity of locomotor CPGs in mudskippers and the control of locomotor programs for moving in water and on land.

B10 Respiratory brainstem structures mediating the mammalian diving reflex and learned breath holds

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The mammalian diving reflex is a ubiquitous reflex that induces a set of physiological protective changes in response to submerging the head in water. These changes include apnea, bradycardia, and hypertension. Aquatic animals that dive for long periods of time also show strong non-reflexive preparatory responses before diving which suggests a learned control over both breathing and cardiac output. To better understand how the body is able to learn to induce such strong homeostatic changes over its physiology I employ an associative learning paradigm in mice to train them to produce apnea and bradycardia in response to odor cues. Mice are able to reliably produce learned apneas to cues and display a prolonged arousal following the apneic period. We then use pharmacology and electrophysiology in brainstem respiratory structures, as well as the cerebellum, to identify the brain regions controlling this apneic response in both reflexive and learned conditions. We hypothesize that these learned sensory inputs, likely originating from the cerebellum, will converge on the same strong inhibitory regulator of breathing, the Botzinger complex, that is theorized to control a variety of respiratory apneic reflexes. Ultimately, learned breath control is relevant to a large set of important ethological behaviors, such as birdsong, as well as a necessary skill for neonates transitioning from reflexive cries to speech, or reflexive swallowing to coordinated feeding behavior.

B11 Actively frozen - Multiple immobility states revealed by novel patterns of leg muscle activity in *Drosophila melanogaster*

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When an animal detects a threat it must make a split-second choice whether to freeze or flee. Freezing is an adaptive behaviour that avoids predator detection, where animals remain immobile in a heightened state of alertness. During freezing skeletal muscles sustain tension to maintain a rigid posture, sometimes in atypical positions for many minutes at a time. We used calcium imaging to visualise muscle activity of intact Drosophila melanogaster while they froze in response to a threat. A striking novel pattern of muscle activation was observed, consisting of rhythmic pulsing of an undescribed muscle in the distal tibia, perfectly correlated between all the legs of the fly. The muscle pulsing ramped up to and preceded movement onset, and we hypothesis that it constitutes a 'preparedness' state for movement. Two modes of muscle activity were apparent. In one, muscles pulsed continuously throughout freezing; in the other muscles were initially silent before becoming active prior to movement onset. Individual flies can show both types of activity; however changing characteristics of the threat (such as salience) biases the flies towards showing one response or the other. These results intriguingly suggest that different muscle pulsing states are indicative of different internal states of the fly, and hint that freezing may encompass a variety of behavioural modes. We now aim to use muscle pulsing dynamics to build a predictive model of fly movement onset, giving insights into the physiological relevance of this phenomenon, and unravelling important general principles about how immobility can be achieved, maintained, and ultimately broken.

EVOLUTION AND DEVELOPMENT I

C1 The scorpion: A novel preparation for understanding the evolution and roles of the biogenic amines

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There is a clear need for studies of biogenic amines in the Chelicerata, especially because they occupy a key position at the base of the arthropod phylogeny. A better understanding of aminergic modulatory systems in the Chelicerata would provide unique and valuable insights into the evolution of those systems in arthropods, because chelicerates may retain ancient features of the arthropodan CNS that have been subsequently lost or modified in other subphyla. To address this need, we have conducted a broad structural and functional examination of several aminergic systems in the Arizona bark scorpion, *Centruroides sculpturatus* (Scorpiones: Buthidae). This species was selected not only as a representative of an important yet understudied chelicerate lineage, but also because its nervous system is highly amenable to physiological manipulations, and it is one of the few chelicerates for which genomic data are available. Using immunocytochemical techniques, we localized neurons in the scorpion ventral nerve cord that contained serotonin (5-HT), octopamine (OA), norepinephrine (NE), and tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis. In doing so, we present the first description of catecholaminergic and octopaminergic systems in the order Scorpiones, and greatly expand upon the preliminary descriptions of the scorpion serotonergic system initially reported by others. Furthermore, our results using analytical chemistry and electrophysiology challenge the commonly accepted notion that NE and epinephrine (E) are exclusively derived features of the Vertebrata and are therefore absent in invertebrates. These findings inform future studies of behavior in the Chelicerata and the evolution of aminergic signaling across bilaterian animals.

C2 Plasticity in the visuo-motor system in embryonically generated monocular *Xenopus laevis*

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Visual inputs through the two eyes are relayed to bilateral central target sites, which jointly permit the formation of retinotopic maps, detection of spectral, contrast, and motion cues and the activation of visuo-motor behaviors. In frogs, retinal ganglion cell (RGC) axons from each eye completely cross at the optic chiasm and target the contralateral pretectum and optic tectum. Symmetric ontogenetic establishment of bilateral circuits is influenced by the availability of visual inputs from both sides. Embryonic addition of sensory inputs from transplanted third eyes in frogs demonstrated an anatomical integration into existing retinotectal circuits. Conversely, targeted ablation of a tectal hemisphere during early development resulted in an innervation of the remaining tectal hemisphere by the canonical contralateral as well as ipsilateral RGCs. To further explore the ontogenetic plasticity of the visual system, we unilaterally ablated visual inputs by excising the optic vesicle at early embryonic stages, which prevents the formation of the eye and central projections. Dye tracings in tadpoles 5 weeks post-manipulation revealed bilateral RGC projections from the singular eye into the ipsi- and contralateral pretectum and optic tectum, confirming a rewiring of the retinotectal circuit. If this projection profile causes altered visuo-motor reflex patterns or induces homeostatic adjustments that compensate for the loss of one eye is currently under investigation. Initial results indicate comparable performance with respect to two-eyed tadpoles and highlight the integrity of this behavior despite circuit manipulations. These data suggest either a complete compensation for this circuit disruption, or the absence of binocular crosstalk in visuo-motor reflexes in *Xenopus.*

C3 Separate and distinct evolutionary paths to spatial vision in chitons

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Animals with distributed light-sensing systems have body-spanning arrays of numerous photoreceptive organs. Recent findings indicate distributed light-sensing systems not only allow animals to detect visual cues (spatial resolution), but also to locate them (spatial vision). How and why does spatial vision evolve in animals with distributed light-sensing systems? We are exploring these questions in chitons (Mollusca; Polyplacophora), nearly all of which have thousands of multicellular sensory structures, termed aesthetes, embedded in their shell plates. In species such as *Chiton tuberculatus*, the aesthetes are associated with clusters of photoreceptors and pigmented cells (eyespots). In other species, such as *Acanthopleura granulata*, the aesthetes are interspersed with image-forming eyes that have lenses made of shell material (shell eyes). From a phylogenetic perspective it appears eyespots and shell eyes evolved separately in chitons. Notably, the smaller, less complex eyespots do not appear to be evolutionary precursors of the larger, more complex shell eyes. Instead, eyespots and shell eyes represent fundamentally different approaches to vision. In behavioral trials, *C. tuberculatus* demonstrates spatial vision by orienting to isoluminant visual cues, but does not use spatial information to inform its defensive responses. In contrast, *A. granulata* uses spatial resolution to inform its defensive responses, but has yet to demonstrate spatial vision. Given differences we have found between their light-sensing organs, light-influenced behaviors, and neural architectures, we propose *C. tuberculatus* and *A. granulata* integrate and process visual information in different ways, highlighting the importance of comparative approaches to the study of distributed visual systems.

C4 Evolution of vision in elephant fishes (*Mormyridae*) – why subtle differences matter

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The family Mormyridae is well known for its active electrolocation, which is also the most studied sensory system in these fishes. This study focuses on another sensory system that has often been deemed neglectable in these fishes: namely, vision and its relevance to their sensory ecology. The visual system of nine different species of mormyrids from the Sanaga River in Cameroon was investigated at the molecular and morphological levels. Retinal transcriptomes revealed that all the Cameroonian species expressed rhodopsin-1 rod opsin (RH1) and three cone opsin genes - two longer-wavelength sensitive LWS genes and one short-wavelength sensitive SWS2 gene. Interestingly, the LWS1 opsin gene was dominantly expressed in all studied species, whereas the LWS2 gene (an ancient gene copy originated before teleost diversification) was found marginally, together with the SWS2. The expression profiles of different species have slightly diversified in evolution mostly involving the proportions of the three cone opsin genes in the retina. Retinal wholemounts were used to assess the density and distribution of ganglion cells across the retina. Ganglion cell topography patterns varied between species. Some species possessed an area of high cell density in the central part of the retina sometimes accompanied by a tendency towards a horizontal streak, while others had a high density of cells ventrally. A single species displayed a unique nasal-dorsal high-density area. These results confirm that the visual system of mormyrid is relatively well-developed and suggest that they probably rely more on vision than previously thought. We discuss how the interspecific variability observed may relate to different ecological strategies.

C5 Evolutionary divergence of locomotion in two related vertebrate species

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Locomotion exists in diverse forms in nature. However, it is often difficult to experimentally understand how closely related species with similar neuronal circuitry can evolve different navigational strategies to explore their surroundings. Here, we investigate this question by comparing divergent swimming patterns employed by two closely related larval fish, *Danionella cerebrum* (DC) and zebrafish (ZF). We show that DC engages in long continuous swimming events when compared with the short burst-and-glide swimming events in ZF. We identify mesencephalic locomotion maintenance neurons in the midbrain to be sufficient to cause the increase in swimming. Moreover, we propose that the observed differences in the swim pattern could provide selective advantage with respect to the availability of dissolved oxygen and timing of swim bladder inflation. Our findings uncover the neural substrate underlying the divergence of locomotion and its adaptation to the environmental constraints.

C6 Evolution of an olfactory subsystem and its link with the multiple emergences of eusociality in Hymenoptera

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Eusociality, one of the most remarkable form of animal society, is primary characterized by a reproductive division of labor where altruistic individuals (workers) forego their own reproduction to help the reproductive individuals (queens) and their progeny. These altruistic behaviors have evolved through kin selection, as helping closely related individuals promotes the indirect transmission of helpers' genes even though their direct reproductive output is null. The fitness benefit of altruism is however lost when directed toward non-relatives and so the ability to discriminate kin from non-kin appears pivotal for the emergence of the eusociality. Hymenoptera gather the highest number of eusocial species with multiple emergences unmatched in other taxa, suggesting that certain factors have facilitated evolution toward eusociality. Ants and hornets, both eusocial, are thought to possess an olfactory subsystem dedicated to the detection of nestmate odors, the cuticular hydrocarbons (CHC). CHC are detected within specific structures on the antenna called basiconic sensilla. They house olfactory sensory neurons whose axons project to a distinct region of the antennal lobe, the primary olfactory brain center. Since ants and hornets have independently acquired eusociality and are phylogenetically distant within Hymenoptera, we investigated the evolutionary origins of this subsystem and its possible link with the multiple emergence of eusociality. To this end, we carried out a comparative neuroanatomical study of a broad sampling of Hymenoptera. We obtained data suggesting that the subsystem is present in social as well as solitary Hymenoptera and which show multiple convergences of olfactory structures accompanying the advent of eusociality.

AUDITORY SYSTEM AND ACOUSTIC SIGNALING I

D1 A comparison of patterns in dolphin whistles with human conversational structure

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In social contexts, bottlenose dolphins (*Tursiops Truncatus*) produce a complex set of acoustic signals that are still poorly understood. These include burst pulses, echolocation clicks and whistles: the latter are single-frequency modulated sounds, and are sometimes learned, individually distinctive whistles that convey the identity of the whistle owner. We refer to these as signature whistles. Whether the vocalizations emitted by dolphins show a grammatical structure, a system analogous to human syntax, is still unknown. We addressed this open question using a data mining approach on a large dataset of their vocalizations and videos, collected from 5 dolphins, in a unique environment, the Dolphin Reef in Eilat, Israel. The Reef combines a natural environment with the possibility of observing and recording the same dolphins over long periods of time.

Previous work on this dataset has allowed us to automatically extract and categorize signature whistles into clustered categories. These categories capture the stable frequency contours even in the presence of a significant intra-whistle variability. Using these data we focus on analysing sequences of whistles to uncover patterns. To do this, we employ probabilistic models (Markov Chain Models) as well as mathematical tools (Network Theory). We draw similarities and differences between the structure that emerges from this analysis with the structure of conversational patterns in humans, as well as in other animal species. In addition, we explore the relationship between categories of whistles and their occurrence at specific positions in the sequence. This study represents a first step towards uncovering patterns in the complex communication system of dolphins.

D2 The bat cerebellum and its roles in vocalization and hearing

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Echolocating bats have been used as a relevant example in neuroscience to understand auditory processing and adaptive behaviour mechanisms. Though studies dealing with bat auditory networks are abundant, research outside of the auditory system of these animals is scarce. Here we present our findings from neurophysiological investigations of the vermis of the bat cerebellum. Previous studies had shown that the vermis of the bat cerebellum is a brain region implicated in sensorimotor integration, orientation, and auditory processing. We tested this idea in our experiments by investigating cerebellar responses to auditory stimuli across the cerebellar vermis through single-unit and field potential recordings in anaesthetized fruit-eating bats (species *Carollia perspicillata*). In addition, we measured neural activity in awake, head-fixed, vocalizing bats that emitted sounds of their own volition. Since characteristics of the mammalian brain are preserved across species, we believe our study could shed light on the role of the mammalian cerebellum for vocalization and hearing. Moreover, the results of this study could bring us a step closer to understanding the cerebellar function and the neurodegenerative diseases that affect it.

D3 Population coding of multi-wavefront echoes by the big brown bat inferior colliculus

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The big brown bat, *Eptesicus fuscus*, shows high-resolution sonar imaging of targets and obstacles in its environment. By listening to the arrival time of echo returns, bats can determine the distance and depth structure of sonar objects. Spectral features of echoes provide cues about the texture and shape of targets. Big brown bats hunt flying insects with multiple, closely spaced reflective surfaces, such as the body and wings. These closely spaced reflective surfaces return multi-wavefront echoes, which overlap to create spectral interference patterns that may convey information about target shape. Previous behavioral studies have shown that bats convert spectral interference patterns into the underlying range separation of reflective surfaces. However, bats do not reliably discriminate the spectral interference patterns created by surfaces 0.5 to 3 cm apart, even though the spectral profiles of these stimuli are distinct. This suggests that the spectrotemporal patterns created by overlapping echoes across this span of echo delay separations are either 1) not well resolved by the auditory system and are encoded as similar stimuli; or 2) bats can discriminate the features of these echoes but treat them as similar. Past neurophysiological recordings in the bat auditory system have not sampled a large enough population of neurons to address the first question, and here, we bridge this gap by characterizing neural responses to multi-wavefront echoes using high-density recordings (Neuropixels 2.0) to capture simultaneous responses from neurons in the Inferior Colliculus. We focus on the dynamic response properties of diverse neurons to understand the interplay of temporal and spectral stimulus coding that contributes to the discrimination of complex auditory objects.

D4 Selective down-regulation of voltage-gated K+-channels in the naked-mole rat sound localization circuit

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Naked-mole rats (NMRs) live in eusocial colonies underground. They are well known for their aging abilities and their adaptations to the low oxygen environment. Less is known about how their ascending auditory systems is adapted to the underground and social living conditions. NMRs have elevated hearing thresholds and long integration times for binaural sound cues. On the other hand, NMRs heavily rely on auditory cues when they communicate with each other using vocalizations. We investigated to what extent these functional adaptations of the NMR hearing are reflected in the distribution of voltage-gated K+-channels in auditory brainstem nuclei. Generally, different types of voltage-gated K+-channels are abundantly expressed in neurons of the binaural auditory brainstem. While high threshold K+-channels (Kv3.1b, Kv3.3) enable neurons to fire at very high frequencies, low threshold K+-channels (Kv1.1) decrease postsynaptic integration times for temporal precise integration of binaural excitatory and inhibitory inputs. Using standard immunohistochemistry against these K+-channels with subsequent confocal microscopy and image analysis we found that high and low threshold K+-channels are present in the NMR auditory brainstem, however with different quantities when compared to other rodent brains. While the low threshold Kv1.1 was similarly expressed in neurons of the medial and lateral superior olive, the high threshold K+-channels Kv3.1b and Kv3.3 were downregulated in neurons of the binaural auditory brainstem when compared to other mammals. The observed downregulation of the high threshold K+ channels either may compensate elevated hearing thresholds or may be a reflection of decreased energy consumption as an adaptation to the low oxygen environment.

D5 Acoustic context modulates natural sound discrimination in auditory cortex through frequency-specific adaptation

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Sound discrimination is essential in many species for communicating and foraging. Bats, for example, use sounds for echolocation and communication. In the bat auditory cortex there are neurons that process both sound categories but how these neurons respond to acoustic transitions, i.e. echolocation streams followed by a communication sound, remains unknown. Here, we show that acoustic context -a leading sound sequence followed by a target sound- changes neuronal discriminability of echolocation vs. communication calls in the cortex of awake bats of both sexes. Non-selective neurons that fire equally well to both echolocation and communication calls in the absence of context become category selective when leading context is present. On the contrary, neurons that prefer communication sounds in the absence of context turn into non-selective ones when context is added. The presence of context leads to an overall response suppression, but the strength of this suppression is stimulus-specific: suppression is strongest when context and target sounds belong to the same category i.e. echolocation followed by echolocation. A neuron model of stimulus-specific adaptation replicated our results *in silico*. The model predicts selectivity to communication and echolocation sounds in the inputs arriving to the auditory cortex, as well as two forms of adaptation: presynaptic frequency-specific adaptation acting in cortical inputs and stimulus-unspecific postsynaptic adaptation. In addition, the model predicted that context effects can last up to 1.5 s after context offset, and that synaptic inputs tuned to low-frequency sounds (communication signals) have the shortest decay constant of presynaptic adaptation.

↑UP

D6 Song duels adhere to context-dependent rules in nightingales

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Adapting vocal outputs depending on contexts is an essential feature of interactive vocal exchanges, like complex human conversations. We investigated the context-dependent flexibility of nightingales, birds with a repertoire of up to 200 songs which perform sophisticated song duels against conspecifics. To test whether song performance is depending on the social context, we exposed wild nightingales to different playbacks. We found that both temporal and spectral aspects of song duels were adjusted depending on the playback familiarity. Specifically, nightingales reduced song duration, increased pauses and reduced the amount of overlaps with playbacks when singing against the bird's own songs (BOS). Using a deep learning approach, we found that playbacks elicited song-matching, during which the bird responded with the same song it just heard. This behavior was increased in response to BOS indicating that familiar songs have the potential to trigger a specific vocal response. During the BOS presentation the birds also performed song anticipations during which they predicted the next song of the playback. This suggests that nightingales sing a predefined sequence of songs which can be evoked by external auditory input. Next, we wanted to study the neural substrates of song-matching behavior by performing intracellular recordings in the premotor HVC of hand raised and song-tutored nightingales while exposing them to song playbacks of different familiarity. HVC neurons selectively responded to the presentation of songs of the bird's repertoire. Taken together, these results show nightingales' ability to adjust vocal outputs depending on the context, and the presence of precise auditory integration necessary for song-matching in premotor neurons of the nightingale's brain.

D7 Early-life stress affects Mongolian gerbil interactions with conspecific vocalizations in a sex-specific manner

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Early-life stress (ELS) affects cognition, learning, and emotional regulation[MR1] , in part by disrupting maturation during critical periods (CPs). Despite similarity between CP elements for cognition-related brain regions and sensory cortices, it is unknown whether ELS affects simple sensory perception. Our recent work indicates that ELS impairs auditory perception of temporally-varying features, such as those in vocalizations. Here we tested whether ELS affects behavioral responses to conspecific vocalizations in Mongolian gerbils. To induce ELS, pups were intermittently maternally separated and restrained during a time encompassing the CP for auditory cortex maturation. Animals were tested as juveniles in a Y-shaped arena where speakers at the ends of two arms played either greeting or alarm vocalizations. This design allowed approach and avoidance of the sound. During the alarm call, all females and Control males had a positive approach metric, suggesting interest in the sound. In contrast, ELS males had a negative approach metric, which was significantly lower than Control males', indicating the alarm call caused avoidance of the sound. During the greeting call, all males and Control females had a negative approach metric, suggesting disinterest in the sound. ELS females displayed great interest in the sound, indicated by a positive approach metric; this was significantly higher than the Control female approach metric. Our results indicate that ELS influences behavioral responses to ethologically relevant sounds in a sex-specific manner.. Such changes may arise from differences in auditory perception, cognition, or a combination of factors. These data are among the first to demonstrate auditory dysfunction emerging from early-life stress.

↑UP

D8 Evaluating phonotaxis variability and selective processing of its underlying neural elements in an insect model

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Selective phonotaxis exhibited by female *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 male, other females respond phonotactically to calls with a wider range of syllable periods. Multiple studies have also provided evidence supporting the importance of prothoracic auditory neurons such as L3/AN2 in the selective phonotaxis of female crickets. We have developed a neuronal model, which includes the contralateral omega 1 neuron inhibiting a source of delayed inhibition as well as a source of selective excitation onto the L3. The inhibition by ON1 is downregulated by Juvenile Hormone III and PTX, which increases selectivity to syllable period, and is upregulated by histamine, which leads to decreased selectivity. Testing this model has included prothoracic injections of JHIII, histamine and PTX followed by an assessment of phonotaxis and L3's selective responses using electrophysiology. The model so far remains valid as we continue to test it. Preliminary data shows the effect of H -7 (a kinase inhibitor) on phonotaxis before and after injection parallels the effect of L3's response. Additionally, exposure to males, decreases selectivity in females. Electrophysiology of L3's response in exposed females is currently being evaluated.

D9 Cortical nucleus mMAN contributes to syllable sequencing in adult Bengalese finches (*Lonchura striata domestica*)

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Birdsong is a learned vocal behavior composed of sequences of individual elements called syllables. As most neuroscience research on songbirds focuses on one species with relatively stereotyped songs, the zebra finch, the neuronal mechanisms underlying the formation of songs from variable syllable sequences remain poorly understood. Here, we test whether cortical nucleus mMAN (*medial magnocellular nucleus of the anterior nidopallium*) contributes to the variable sequencing of adult Bengalese finch (*Lonchura striata*) song. mMAN is part of a basal ganglia-thalamo-cortical loop that projects to motor nucleus HVC. Bengalese finch song contains branch points, where one syllable can be succeeded by multiple following syllables in a probabilistic manner, and chunks, where multiple syllables are sung in stereotypical order. After mMAN lesion, sequencing became more random: 1) Transition probabilities at branch points became less predictable (for example ab-c 70% and ab-d 30% became ab-c 60% and ab-d 40%), characterized by an increase in total transition entropy. 2) We observed breaking of previously stereotyped chunks and introduction of new transitions between syllables. 3) Repeat phrases, where the same syllable is repeated multiple times, increased in length and variability after the lesion. These changes were apparent as soon as singing resumed after the lesion and persisted after the song had stabilized. mMAN lesions in adult zebra finches have previously been found to have little influence on song production. In contrast, our results suggest that nucleus mMAN contributes to the variable sequencing of Bengalese finch song, and suggests that models of song production may need to include areas upstream of premotor song nucleus HVC for species with more complex song syntax.

D10 Filling in the gaps: auditory processing by descending neurons in a bush cricket

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Numerous insect species exhibit surprisingly complex behaviours and accurate perception. Many orthopterans are notable for their conspicuous acoustic communication. A duetting bush cricket, *Ancistrura nigrovittata*

(Phaneropteridae), provides one of few well-studied models of auditory processing. Males sing with a distinct temporal pattern at ca. 16 kHz and females respond after 30 - 50 ms with a short click at ca. 28 kHz. The critical parameters for song recognition are known and six types of prothoracic neurons involved in auditory processing have been studied in detail, while descending neurons (DNs) remained fairly overlooked. Data on DNs from other bush crickets are fragmentary as well. We ask if DNs might contribute to song recognition. Our data from intracellular recordings and stainings reveal a diverse group of DNs. This is apparent in the morphology of the neurons as well as in their responses to acoustic and vibratory stimuli. Functionally, this cluster contains purely vibratory and purely auditory neurons, as well as ones that respond to both modalities. Within the auditory DNs, temporal and frequency tuning vary greatly. Neurons tuned to the male song frequency are especially interesting, as they could explain the short-delay response of the female without involving brain pathways. In addition, neurons responding to both sound and vibration could participate in the switch from acoustic long-distance communication to acousto-vibratory short-distance communication. Further morphological studies including thoracic projections, physiological characterisation and immunohistochemistry may help to elucidate the neural basis of mating behaviour in this model organism.

D11 Dual region recordings in the sound localization pathway of barn owls to investigate stimulus selection of salient stimuli

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Barn owls are specialists in sound localization. Their well-described midbrain stimulus selection network, a circuit containing a map of auditory space dedicated to localizing salient sounds, provides a unique opportunity to study the flow of information between midbrain and forebrain, where a transformation of coding scheme is indicated by the vanishing of a map of auditory space between midbrain and forebrain regions. This project worked towards investigating how the circuit conducts bottom-up relay for salient stimuli in environments with competing sounds. Earlier in vivo recordings in the owl's optic tectum (OT) have shown that gamma oscillations are spatially tuned to both visual and auditory information, and may play a role in stimulus selection. However, previous recordings in deep midbrain structures, like OT, have relied on single electrodes in a single region and an open question remains regarding how oscillations contribute to information flow during stimulus selection. Towards this end, we recorded spike responses and local field potentials in OT and one of its downstream forebrain regions simultaneously in anesthetized owls. So far, we observe heterogeneity in tuning properties to binaural cues in the forebrain, ranging from peaked tuning curves to broad tuning to contralateral space. However, while tuning may differ between brain regions, firing rates positively correlate both during spontaneous activity in the absence of stimuli and during presentation of stimuli, suggesting connectivity. Future experiments will determine the role of gamma oscillations in promoting stimulus selection during presentation of two competing sounds, and determine whether gamma power is predictive of bottom-up relay towards salient stimuli during sound orientation behavior.

D12 Structure and function of the cochlear nucleus of the naked mole-rat

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Background: Sensory input shapes the formation of brain circuits, the essence of which has been most extensively studied using experimental sensory deprivation. This project takes advantage of a unique model species, the naked mole-rat (Heterocephalus glaber), that naturally exhibits elevated auditory thresholds, poor frequency selectivity, and limited sound localization. Our study is to examine if these poor hearing abilities influence structure and function of the auditory nerve synapses in the cochlear nucleus.

Methods: Serial section transmission electron micrographs were used to reconstruct auditory nerve synapses in 3D. In addition, auditory brainstem measurements (ABR's) were taken, and immunohistochemistry was used to analyze the distribution of voltage-gated potassium channels.
Results: Naked mole-rat endbulb synapses did not exhibit the usual deafness-associated changes as previously reported for animal models of auditory deprivation or deafness. These data suggest that there may be central compensation for the reduced hearing levels. Immunohistochemical results are still under investigation.
Conclusion: Current findings suggest that naked mole-rats, despite their elevated auditory thresholds, do not exhibit changes in synaptic ultrastructure in endbulbs characteristic of congenitally deaf animals. This result suggests that the reduced auditory abilities of naked mole-rats have a diminished impact in the cochlear nucleus. These findings may help identify potential protective mechanisms in the naked mole-rat against the effects of poor hearing.

D13 Sound localization in chickens

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Birds – except auditory specialists such as the barn owl – inspect and interact with the environment mainly relying on vision. However, sound localization is also crucial for their survival and reproduction, e.g., by locating conspecific and predator calls. In order to investigate the sound localization accuracy and how it is affected by sound duration, we conducted a behavioral experiment where 3 chicken males performed an azimuthal sound localization task with broad-band noise, using a 2-alternative forced choice paradigm. We determined the minimum audible angle (MAA) as measure for localization acuity. The chickens had a high localization acuity compared to other auditory generalist bird species tested so far, and were better at locating long-duration stimuli (1 s; MAA=16°) compared to brief ones (0.1 s; MAA=26°). Moreover, the head position during localization was monitored, showing that chickens seem to keep the head oriented towards the central reference position, rather than actively orienting the head towards the target stimulus. These findings – considering also recent discoveries about the neural representation of auditory space in the midbrain – suggest that the chicken might use auditory cues to map the environment, most likely integrating them with the more accurate visual inputs.

D14 Cricket singing behaviour – the role of abdominal ganglia

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Although field crickets use rhythmic opening and closing movements of their front wings to generate the pulse and chirp pattern of their songs, the abdominal ganglia house the neuronal network of the calling song central pattern generator. We compared the effects of specific lesions to the abdominal connectives on calling song activity in four different cricket species with very different calling patterns. In all species singing activity was abolished after the connectives between the metathoracic ganglion complex and the first abdominal ganglion A3 were severed. The song structure was lost and males generated only single sound pulses when connectives between A3 and A4 were cut. Severing connectives between A4 and A5 had no effect in a trilling species, it led to an extension of chirps in a chirping species and to a loss of the phrase structure in two *Teleogryllus* species. Cutting the connectives between A5 and A6 caused no or minor changes in singing activity. In spite of the species-specific patterns of calling songs, our data indicate a conserved organization of the calling song motor pattern generating network with the generation of pulses controlled by ganglia A3 and A4, while A4 and A5 provide the timing information for the chirp and/or phrase structure of the song. For rivalry and courtship singing in *G. bimaculatus* lesions to the abdominal ganglia revealed that rivalry singing is organised in a similar way than calling song. In contrast courtship song fails if the connectives between A5 and A6 are severed, indicating that the network controlling courtship song is organised in a different way and located more posterior than for calling and rivalry song.

D15 Bats call anti-phase to rhythmic noise: dynamic time-domain jamming avoidance in freely socializing bats

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Bats live and navigate in noisy environments, where acoustic signals are commonly masked or degraded by ambient noise. While acoustic jamming avoidance in the spectral domain has been documented in several bat species, less attention has been paid to temporal-domain acoustic jamming avoidance. In this study, we presented groups of freely socializing *Carollia perspicillata* bats with amplitude modulated white noise that masked significant portions of the frequency band used for both echolocation and social communication. We found adult bats spontaneously clustered the timing of their vocalizations to fall predominantly within the amplitude troughs. In addition, the number of emitted vocalizations decreased in proportion to the degree of spectral masking incurred by the noise. Our findings suggest that bats dynamically adapt temporal parameters of their vocalizations to preserve signal quality in the presence of rhythmic, predictable ambient noise.

D16 Neural representation of conspecific communication sounds in the frontal auditory field of the Mexican free-tailed bat

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In this study, we examined the auditory responses of a prefrontal area, the frontal auditory field (FAF), of the bat *Tadarida brasiliensis* and present a comparative analysis of the neuronal response properties with the primary auditory cortex (A1). We compared single-unit responses from the A1 and the FAF elicited by species-specific vocalizations. Although most FAF neurons responded to more than one communication call, call-selectivity was greater in FAF versus A1. We calculated the neuronal spectro-temporal receptive fields (STRF), using dynamic moving ripples, to determine whether we could predict responses to the communication calls and then provide an explanation for the differences in call selectivity between the FAF and the A1. In the A1, we found a high correlation between predicted and evoked responses. This indicates that processing in the A1 was essentially linear and their functional properties can be explained by their STRF. However, we did not generate a reasonable in the FAF and, thus, it was not possible to predict the responses to the communication calls. This indicates that apparently there are non-linear response properties stronger than the linear response properties extracted by the STRFs generated by the dynamic moving ripples in the A1. Finally, we present data in response to a constructed sequence of communication calls to assess if neural suppression characteristic of the A1 is also present in the FAF. These data are consistent with a role for the FAF in assessing distinctive acoustic features downstream of A1, similar to the role proposed for primate ventrolateral prefrontal cortex.

D17 Neural underpinnings of speciation by reinforcement in chorus frogs: the mystery of mismatch between temporal tuning and advertisement call structure in allopatric, but not sympatric populations

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Diversification of reproductive communication behaviors can occur within a species when different populations interact with different heterospecifics, generating divergent selection pressures across geography. Consequently, unique male call properties and female mating preferences may evolve within (sympatry) compared outside of (allopatry) these species contact zones. Chorus frogs, *Pseudacris feriarum,* show divergence of call traits (pulse number and rate) across populations; for example, advertisement

(Adv) calls of sympatric frogs from Georgia and South Carolina have faster pulse rates (PRs) and/or more pulses, respectively, relative to an allopatric population from Alabama. To identify the neural correlates of divergence in call preference, we made extracellular recordings from auditory midbrain neurons (AMNs) while presenting: (a) acoustic stimuli that varied in their temporal properties and (b) natural Adv calls from each population. We tested the hypothesis that the temporal tuning of Interval Counting Neurons (ICNs), which respond only after a threshold number of pulses, would be rescaled to PRs typical of the Adv calls of each population. While the selectivity of ICNs in GA (sympatric) frogs were well matched to pulse rates in their calls, ICNs in the allopatric group showed tuning to faster PRs than those seen in their Adv calls and were better matched to the PRs of their aggressive calls. These results suggest that the latter frogs do not use ICNs for Adv call recognition. Instead, allopatric groups may represent a pleisiomorphic condition in which long-interval neurons (LINs), tuned to slow PRs, are used to recognize Adv calls; the divergence in sympatric populations may, therefore, involve repurposing and re-wiring LINs and ICNs.

ELECTROSENSORY SYSTEM I

E1 Electrocommunication and steroid hormone production vary with social context across electric knifefishes

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Communication is dynamic, and signal use varies by social structure, sex, reproductive condition, and context. Hormones, including gonadal steroids and glucocorticoids, regulate social signaling across vertebrates. How variation in signal use and hormonal responses to social interaction covary with sociality, however, is less explored. Weakly electric knifefishes are an excellent model for investigating hormonal control of communication. Knifefish produce electric organ discharges (EODs), whose frequencies are modulated during aggression and courtship to produce signals called chirps. We examined chirping during overnight recordings in isolated fish, same-sex pairs, and opposite-sex pairs in three species that vary in social behavior: territorial *Apteronotus albifrons*, semi-social *Apteronotus leptorhynchus*, and gregarious *Adontosternarchus balaenops*. We also measured gonadal steroid and cortisol levels before and after social housing. Chirping was sex- and context-specific, but variation in chirp rates was not always consistent with previous findings from artificial playback experiments. Although artificial playback studies found no sex differences in chirp rate in *A. albifrons*, chirp rate was higher in males than females in live overnight interactions. Also, in territorial *A. albifrons*, hormone levels did not vary directly with social context, but cortisol responses to social interaction were condition dependent. Gregarious *A. balaenops*, however, were more stressed in isolation. These results suggest social and experimental context influence the expression of sex and species differences in signaling and physiological responses to a social challenge.

E2 Activity patterns in a wild population of the electric fish *Apteronotus macrostomus* and *Eigenmannia sp.*

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Signal processing in the electrosensory system of electric fish has been extensively studied in laboratories, but little is known about behavioral relevant stimuli they actually process. Field studies in natural habitats are needed to identify the sensory environment, they evolved to. Electric fish continuously emit an electric field that can be used to track their behavior without disturbing the fish. We distributed 16 electrodes along a line within a 20 m section of a side arm of the Rio Canocamoa near San Martin, Colombia, covering a variety of micro habitats including stones and roots as suitable resting sites and sections of sandy riverbeds void of hiding places. We continuously recorded the individual specific electric signal of two species, *Apteronotus macrostomus* (of the *Apteronotus leptorhynchus* species group) and *Eigenmannia sp.,* for 4 consecutive days outside the breeding

season (October 2019). Species were identified based on EOD frequency and waveform. Only a few solitary *Eigenmannia sp.* (n=3) were traversing the recording section and a single one stayed stationary. Most of the fish we detected were of the species *A. macrostomus* (n=154). All of them were resting between stacked stones in the shallow parts of the stream with faster flowing water (84 %) and only for short periods of time (18.9 min ± 37.9 min) they explored the vicinity of their resting site within 4.4 m ± 1.8 m. The overall movement activity was low but increased in the evening and was highest during the night. At dawn the population activity decreased again and was significantly lower during the day than during the night. We conclude that outside their breeding season the stone microhabitat provides sufficient shelter as well as enough food for the quite abundant *A. macrostomus*.

E3 Identifying stereotyped movement patterns during social interactions in weakly electric fish

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Localizing the source of a signal is key in guiding the behavior of the animal successfully. Localization mechanisms must cope with the challenges of representing the spatial information of weak, noisy signals. Comparing these strategies across modalities and model systems allows a broader understanding of the general principles shaping spatial processing. The electrosensory system provides an advantageous comparison because it has a spatially mapped architecture right from the periphery -like the visual system- but it must also compute the location of conspecific -like the auditory system- since these signals activate the entire receptor array. In order to understand the neural mechanisms responsible for localization of conspecific signals in the electrosensory system, we first aim to have a quantitative understanding of the spatio-temporal structure of these signals during natural behaviors. Complex behaviors are typically composed of stereotype bouts of movements. Ethograms can be used to identify these stereotyped patterns and their sequence and modern computational tools permit a rigorous quantitative approach to identifying stereotyped behaviors and their sequence. Data on the relative motion of interactive fish were captured in a variety of social context. We used a cutting-edge analysis technique that leverages a combination of supervised-learning and unsupervised-learning neural network, dimensionality reduction, and cluster analysis, to identify stereotyped patterns of movements during these social interactions. Identifying these stereotype patterns allows use to characterize the structure of these behaviorally important sensory signals.

E4 Characterization of the agonistic behavior of the weakly electric fish, *Gymnotus sylvius*

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Agonistic behavior is the set of behavioral displays that resolve conflict situations among conspecifics. Animals compete for different and limited resources as food, territories, mates, breeding sites, etc. Comparative studies among sympatric related species are relevant to understanding how agonistic behavior has evolved. The richness of locomotor and electric agonistic displays together with the knowledge of their circuital substrate and modulators make weakly electric fish especially suited for neuroethological comparative studies. Although all species of the genus *Gymnotus* are considered aggressive and territorial, *Gymnotus omarorum* is the only South American species in which the agonistic behavior has been carefully addressed. Little is known about the natural history and social behavior of *Gymnotus sylvius,* which occurs in sympatry with *Gymnotus omarorum* in freshwater environments of Argentina. We describe the agonistic behavior of *Gymnotus sylvius* in dyadic encounters in laboratory settings. All dyads of *Gymnotus sylvius* engaged in agonistic interactions that ended in the establishment of dominant-subordinate status. Surprisingly, the dominant of each dyad was always the first fish to attack, not the larger fish. In addition to the locomotor displays, submissive transient electric signals were registered, as offs (interruptions of the Electric Organ Discharge, EOD), and chirps (brief increases of the EOD rate). Dominant fish increased theirEOD rate, whereas subordinate fish maintained the initial EOD rate. As a starting point for future comparative studies, we highlight the conserved aspects of the behavioral displays of *Gymnotus sylvius* with those previously reported in *Gymnotus omarorum* and speculate on the evolutionary implications of the differences found.

E5 Population coding of spatial information in the electrosensory system during social interactions

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Localizing the source of a signal requires sophisticated neural mechanisms and we are still uncovering the coding principles that support accurate spatial processing. Weakly electric fish can detect and localize distant conspecifics, but the way this spatial information is encoded is unclear. Here, we investigate the spatial representation of conspecific signals in the hindbrain to determine how the properties of the heterogenous population of pyramidal cells affect the spatial coding accuracy of conspecific signals. We hypothesize that specific subset of cells provides more accurate spatial information about conspecific location. We stimulated the fish with an artificial signal that replicates both the spatial and temporal structure of conspecific signals. We recorded from cells with various receptive field positions covering the entire body surface and analyzed the spike train with spike-train distance metrics to determine how accurately the location of the stimulus is encoded. We found that some pyramidal cells (such as ON-type, and those within the deep layer) encode the spatial information more accurately while other sub-groups (OFF-type, and superficial layer) provide less accurate information. Our results help us understand how the heterogeneity of a population of cells allow the efficient processing of signals and suggest that a segregation of the spatial information stream starts earlier in the sensory pathway.

E6 Characterisation of a new class of aerial electroreceptor in bees

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In recent years, studies have described a new sensory ability employed by bees that allows them to detect and respond to external electric fields generated by objects in their environment. This sense, termed aerial electroreception, has been shown to play a role in foraging and intraspecific communication. Until now, the mechanosensory hairs of bumblebees, *Bombus terrestris*, and antennae of honeybees, *Apis mellifera,* have been proposed as the putative sensory structures enabling electroreception, operating via the electromechanical deflection of the charged sensory structure. Here, we propose a new class of aerial electroreceptor in bees, the antennal placode sensilla, which are considered to function exclusively as olfactory receptors. Preliminary evidence from experiments using microscanning laser Doppler vibrometry show that placodes exhibit some electromechanical sensitivity to electric fields of biologically relevant frequencies and magnitudes. Furthermore, data from electrostatic force microscopy show that the placode membranes of honeybee drones hold a quasi-permanent electrostatic charge that differs from the charge of the surrounding antennal cuticle and is maintained long after death. This evidence, combined with the high neural investment of placode sensilla, strongly suggests bimodal sensing of olfactory and electric field stimuli by these sensory units. Using these multidisciplinary approaches, incorporating biophysical, biochemical and electrophysiological techniques, we hope to shed light on the mechanism by which placode sensilla operate with bimodal sensitivity and enable electroreception in bees.

E7 Sensing the electrical world: modelling electroreception in terrestrial Arthropods

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Arthropods such as bees and spiders have been shown to detect electrical fields (a sense known as electroreception). Whilst this discovery expands our view of how such organisms explore their environment, the exact mechanisms of this curious sense remain unclear. With such new discoveries, many questions arise that require both theoretical and empirical examination. My poster introduces the theory behind the fundamental mechanics of this sensory phenomena and uncovers some of its mechanical and sensory complexities. It will first show the physical, biological, and parametric feasibility of this sense in comparison with aero-acoustic sensing. Secondly, it will illustrate the possible interactions between hairs and electrical fields introducing the concept of a sensitivity contour (regions of the solution space where hairs deflect to a given sensory threshold). Finally, it will establish the sensory possibility of object identification and localisation via mechanoreceptive filiform hairs.

E8 Electrocommunication signals motivation to continue mutual assessment in the electric fish *Apteronotus leptorhynchus*

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Animals across species rival for limited resources. In order to economize competitions animals pursue different strategies, including the development of distinct opponent assessment behaviors or dominance hierarchies. Using cues and communication signals, contestants can gather information about their opponent, adjust their behavior accordingly, and thereby avoid high costs of escalating fights. We tracked electrocommunication signals, movement behaviors, and agonistic interactions of the electric fish *Apteronotus leptorhynchus* in staged competitions. A larger body size relative to the opponent was the sole significant predictor for winners. Sex and the frequency of the continuously emitted electric field only mildly influenced competition outcome. In males, correlations of body size and winning were stronger than in females and, especially when losing against females, communication and agonistic interactions were enhanced, suggesting males to be more motivated to compete. Fish that lost competitions emitted the majority of rises, but their emission rate depended on the competitors' relative size and sex. The emission of a rise could be costly since it provoked ritualized biting or chase behaviors by the other fish. The temporal and quantitative evaluation of physical and communicative interactions in the context of the contestant's physical attributes suggest that *A. leptorhynchus* adjusts its competition behavior according to mutual assessment, where rises could signal a loser's motivation to continue assessment through ritualized fighting. These competitions could be used to define relative dominance between contestants, and thereby their relative access to resources, which reduces the necessity of repetitive, costly fighting and is beneficial for both individuals.

E9 Encoding of communication signals at high beat frequencies in the electrosensory system of *Apteronotus leptorhynchus*

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Weakly electric fish employ their electric field for prey detection and communication. When interacting, the electric organ discharges (EOD) of two fish superimpose and cause regular amplitude modulations, called beats. The frequency of beats depends on the frequency difference between the individual signals. It was assumed that very high beat frequencies (>250 Hz) are not behaviorally relevant since they are not well encoded by the primary afferents of the electrosensory system (P-units). Motivated by field data that clearly show electrocommunication at high beat frequencies, we recently demonstrated that P-unit tuning extends in a repetitive manner way beyond the behaviorally relevant range (>3300 Hz). Thus, in contrast to previous knowledge, high beat frequencies are indeed reliably encoded by the P-units. In the context of beats, the fish further generate short-term frequency excursions as communication signals, so-called chirps. Here we investigate whether and how chirps are encoded at high beat frequencies. We first analyzed P-unit responses and show that chirps are well encoded beyond the previously assumed range. The primary afferents project to pyramidal cells in the hindbrain. In a second step, we perform in-vivo recordings of pyramidal cells in the electrosensory lateral line lobe (ELL). We show preliminary data that suggest that chirp encoding over a wide range of beat frequencies is maintained on the level of the hindbrain as well.

E10 Using an interactive biomimetic fish robot to investigate the role of electric signaling and locomotion during social interactions in groups of weakly electric fish

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Animals that live in social groups exchange information via various communication channels in order to recognize and react to conspecifics. In mormyrid weakly electric fish, individuals continuously emit and perceive pulse-type electric organ discharges (EOD) to establish highly complex social group behaviors. Besides emitting context-specific signal patterns, shoaling mormyrids frequently engage in episodes of interactive electric signaling by synchronizing their EODs to each other. Our previous studies have shown that these fish also interact with an EOD-emitting fish robot. However, it is not fully known which social cues these signals convey, what they mean, and which behaviors they evoke in other fish. Here, we investigate the basic processes that facilitate social interactions in shoals of weakly electric fish by developing an interactive biomimetic electric fish robot ("ElectroFish") that serves as a communication partner. Because during EOD synchronization two fish respond to each other within a short time window of 20-30ms, we developed an automated, on-line EOD localization system using neural networks to allow for real-time identification of the EOD sender. For subsequent directed responses of the robot such as interactive locomotion and EOD synchronization, we also developed a response system that integrates the electric signaling properties into the robot control software. By designing this fully interactive robot, which operates in closed-loop both electrically and locomotorily, we have full control over the cues we inject into the social system. Thus we are able to investigate effects of ensuing locomotor and/or electrical interactions on behavior and understand the rules of information transfer between individuals in shoals of weakly electric fish.

E11 Aerial electroreception and the electric landscape

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Aquatic electroreception has been studied for decades, yet there is also currently a burgeoning research focus on aerial electroreception. Bees can detect and potentially use floral electric cues while foraging, and electric fields can trigger dispersal behaviour in spiders. Furthermore, increasing evidence suggests that mechanosensory hairs, nearly ubiquitous in arthropods, can act as sensors to detect ecologically relevant, quasistatic electric fields. We have identified the tantalising prospect that electroreception is a biogeographically and phylogenetically widespread trait amongst arthropods, along with the realisation that the electric environment contains a wealth of information for these animals. Thus, as with any sensory modality, it is important to consider the sources of both signals and noise in the environment. As such, we present an up-to-date map of the terrestrial and aerial electric landscape using the latest biological, environmental, and atmospheric electrical data, discussing its relevance to different electroreceptive systems including receptor

and behaviour levels. This contributes to providing an ecological context to aerial electroreceptive systems, but also uncovers potential niches for future research into this emerging field of sensory biology.

E12 Weakly electric fish out of (swampy) water – how hypoxia and captivity effect brain cell proliferation and apoptosis in *Petrocephalus degeni*

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Hypoxia as an indirect consequence of climate change will adversely affect freshwater fish species. Fish facing hypoxia commonly show physiological changes that yield trade-offs for brain size, brain plasticity or overall behavior. Weakly electric fish, *Petrocephalus degeni*, inhabit severely hypoxic swamps in Uganda while maintaining a remarkably large brain. We compared brain cell proliferation and apoptosis of cells in fish collected from hypoxic swamps to that of captive fish kept for 10 d under experimental hypoxia or normoxia. The density of cells undergoing proliferation in the telencephalon was quantified using PCNA immunohistochemistry. TUNEL labeling was used for a qualitative and quantitative assessment of apoptosis. Individuals from the hypoxic lab condition showed a significantly lower density of PCNA+ cells and higher rate of apoptosis than those in normoxic conditions, and individuals collected from the field showed the overall highest density of PCNA+ brain cells. Our results show that while P. degeni tolerate severe hypoxia in their natural habitat, hypoxia negatively affects brain cell proliferation and promotes apoptosis.

SOCIAL BEHAVIOR AND NEUROMODULATION I

F1 Interaction between arginine vasotocin and gonadal hormones in the regulation of reproductive behavior in a cichlid fish

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The nonapeptide vasopressin and its teleost homologue arginine vasotocin (AVT) have been associated with the regulation of different aspects of social behavior (e.g., mating and aggression). Given the fact that androgens are also known to regulate reproductive behavior, we hypothesized that AVT and androgens could be interacting, rather than acting independently, in the regulation of reproductive behavior. In the present study, we aimed to understand the effect of AVT and its interaction with gonadal hormones (putatively androgens) on different aspects of reproductive behavior of a polygynous and territorial cichlid fish, the Mozambique tilapia (Oreochromis mossambicus). Using a within-subject design, we treated territorial males, that were previously castrated or sham-operated, with different dosages of AVT as well as with a V1A receptor antagonist (Manning compound) and subsequently analyzed their behavior towards females and towards an intruder male. Our results showed that AVT affected the behavior of territorial males towards females but not towards males. Specifically, AVT-treated males interacted less with females than saline-treated males while both gonadectomized and sham-operated males injected with AVT were less aggressive towards females. Moreover, in sham-operated males, blocking V1A receptors increased the frequency of bites towards females in comparison to saline-treated males, but not in castrated males. This result suggests that AVT down-regulates aggressiveness towards females through the action of V1A receptors in the gonads (putatively decreasing androgen secretion), and that androgens up-regulate this behaviour. In summary, our results suggest that AVT may modulate social behavior, through interaction with gonadal hormones.

F2 Oxytocin modulation of socially driven adult neurogenesis in zebrafish

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Adult neurogenesis, the formation of new neurons from precursors cells, is regulated by both intrinsic and extrinsic factors. The social context is a key environmental factor that can modulate positively or negatively the formation of new neurons. Preliminary work from our lab shows that social isolation has a negative impact on cell proliferation in zebrafish, which can be rescued by the exposure to a social stimulus (a mixed-sex shoal). Moreover, in rats, adult neurogenesis can be modulated by the action of oxytocin receptors present in the hippocampus. Here, we used a zebrafish mutant line for the oxytocin receptor, to test if oxytocin mediates the effects of the social environment on adult neurogenesis in brain nuclei belonging to the social decision-making network, a brain network that regulates social behaviour and is influenced by the action of hormones and neuromodulators, like oxytocin. Our results indicate an effect of oxytocin on brain cell proliferation in Dm, Vc, PPa, PPp, Vd and the pretectum. Thus, this study confirms a key role for oxytocin on the social modulation of adult neurogenesis in vertebrates.

F3 Principles underlying information flow across the entire brain of the zebrafish

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How information is processed and how it flows through the brain to generate motor behaviours and cognitive functions is a paramount question in neurosciences. As early as in 1949 Donald Hebb proposed that individual neurons cooperate to form functional structures (neuronal assemblies) that communicate through phase sequences. Recent experiments support the existence of assemblies but how does the information flow between these neuronal assemblies and how (in case they exist) the phase sequences are, remain elusive. By using zebrafish larva as experimental model in combination with light-sheet microscopy, we monitored whole-brain dynamics with single-neuron resolution while simultaneously recording free tail movement as a behavioral output. I will present the results of an exhaustive characterization of the topology, occurrence frequency and transition probability of whole-brain assemblies and phase sequences triggered spontaneously, or by different sensory stimuli and therefore during different behavioral motives. Results show that there is a large variability of whole-brain assembly characteristics. In addition, there are almost no silent moments in the brain while increases and decreases in the number of active assemblies are more consistent with activation cascades than with sequences in which each has a temporary beginning and end. On top of that it is observed a global state shift in the brain activity characterized by the shutting down of a vast majority of active assemblies at the same time that the inactive assemblies start up. In particular, I will show those shifts associated with the activation of noradrenergic neurons from the locus Coeruleus (LC) studying the differences in the dynamics of assemblies and the sequences topology before and after LC activation.

F4 Social modulation of neuronal complexity in zebrafish

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In response to variation in the social environment individuals can alter their behavioural phenotypes, a phenomenon known as phenotypic plasticity, which is normally based on neural plasticity. Neural plasticity is the ability of the brain to reorganize its neural connectivity in response to environmental changes and might include changes in morphology, in neurophysiological functions, as well as modification in the neural networks. The main goal of this work was to assess the impact of environmental complexity in the dendritic arborization of zebrafish reared in distinct social environments. The complexity of the social environment was induced through variation in group size (small vs. large) and group stability (stable vs. unstable) leading to four experimental treatments (small stable, small unstable, large stable, large unstable). The quantification of dendrites was performed in five different brain areas - Telencephalon, Diencephalon, Optic tectum, Cerebellum, and Brain stem -, using the microtubule-associated protein 2 (MAP2) has a dendritic marker. We found differences in the dendritic density related with the complexity of the social environment, such that animals raised in less complex social environments (i.e. small and stable shoals) present a decrease in their dendritic density, which was paralleled by changes in their social behaviour. Our results indicate the relevance of the social environment in the modulation of neuronal complexity during development which is paralleled by changes in behavioural performance.

F5 Organization of a layered structure in the dorsal telencephalon of gobies

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The caudo-dorsal part of the goby telencephalon displays an extremely conspicuous level of organization that has not yet been described in any other fish family. This neuronal structure consists of small, granule cell like neurons, which are organized into 7 dense layers. Such layered clustering of neurons is known to facilitate reciprocal and parallel information processing in other neuronal networks and thus to provide advanced processing of neuronal input. To investigate the connectivity of this structure with other brain areas, we performed in-vitro electroporations of the structure using neurobiotin. These tracing experiments revealed fibers extending to different divisions of the telencephalon, as well as to two midbrain nuclei. Single-cell morphology and intra-structure connectivity patterns within the layers were visualized by applying the lipophilic tracer DiI to single cells in lightly fixed brain slices. Single cell staining revealed neurons with limited dendritic arborization characterized by numerous spines, resembling granule-cells of the cerebellum. Immunohistochemical experiments showed that some of the cells within the layers express GLS-2 and receive Substance-P and parvalbumin-immunoreactive-fibers. These experiments suggest that the structure is a part of Dl, a homologue to the medial pallium, which includes the hippocampus in mammals. Our data thus provide first evidence to decipher the function of this structure, which we aim to investigate with calcium imaging and patch clamp experiments.

F6 Biophysical properties and gene expression profile of single periaqueductal gray neurons in the mouse brain

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The midbrain periaqueductal gray (PAG) is a longitudinal columnar structure where instinctive behaviours as diverse as escaping from predators, vocalising, and pup grooming segregate onto distinct anatomical subdivisions. This parallel between behaviour and brain circuit anatomy provides a unique opportunity for investigating how neural mechanisms support the computation of different adaptive actions. Here, we characterised the biophysical properties and gene expression profile of single neurons across PAG subdivisions. First, we used loose-seal cell-attached recordings to measure the baseline firing properties of PAG neurons in acute midbrain slices of transgenic mice. We found that, even in the absence of synaptic inputs, inhibitory ($VGAT^+$) neurons fired action potentials spontaneously, whereas excitatory ($VGluT2^+$) neurons were mostly silent. Next, to link the expression of ion channels, receptors, and molecular effectors to specific PAG subdivisions, we performed cell-type specific single-cell RNA-sequencing while preserving the anatomical origin of each neuron. We isolated fluorescently labelled neurons from acute midbrain slices of transgenic mice and processed them with Smart-seq2 and a target sequencing depth of 4 million reads per sample. Unsupervised clustering of the resulting data revealed putative subtypes of neurons that map onto different PAG subdivisions, whereas differential expression analysis identified candidate genes for setting and regulating key biophysical properties of PAG neurons. By leveraging the unique relationship between PAG circuit anatomy and behavioural output, our work uses anatomical location as an anchor to provide a framework for studying how molecularly defined biophysical properties might underpin behavioural control by the PAG.

F7 Exploring density dependent locust marching with immersive virtual reality

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Collective marching across large distances is an astonishing feat exhibited by larval desert locusts, *Schistocerca gregaria*. Locusts coalesce into groups, called bands, which can further merge into larger collectives. Marching is shown to be dependent on locusts' local density. Danger of cannibalism is also a factor maintaining the collective inertia. However, despite observations in various lab and field studies, local interaction rules, and their translation to collective direction selection, are yet to be mapped. Here, we are utilizing a custom-made immersive virtual reality technology to place a focal locust within a virtual marching band of low-poly realistic locust models. We explore the parameter space of density vs coherence with the aim of finding out the least required conditions to initiate and sustain trajectory alignment. Virtual reality system will additionally allow utilizing neurobiological approaches to explore underlying neural correlates of locust marching in near future.

F8 Behavior and neurobiology of attaining social status in cichlids

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Neuroplasticity is the modification of neuron morphology caused by various stimuli to adapt to situational changes. Although social interaction within a dominance hierarchy is a major modifier of neural circuitry, little is known about how neuronal morphology relates to attaining an initial position within the hierarchy. We examine this relationship in juvenile fish of the cichlid *Astatotilapia burtoni*, a species in which males exist in a dominance hierarchy. Once a single dominant male was established within a community, we quantified behaviors and performed Golgi-Cox staining to examine neuron morphology of dominant and subordinate fish. Dominant males showed more physical aggression while subordinates engaged in more non-physical aggression and submissive behaviors. Dominant males also had longer cell processes throughout the brain, and larger cells with more branching in the telencephalon. These data suggest increased synaptic connections may facilitate the behaviors and physiology required for attainment of dominance. This work may lead to better understanding of how neuroplasticity is related to an individual's social rank in hierarchical societies across vertebrates.

F9 The role of the fish amygdala in visually-driven aggressive behavior of Siamese fighting fish

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Aggression is a social behavior that is widespread in nature. However, the sensory modalities that trigger it, vary drastically between species. Organisms tractable to detailed circuit manipulation in the laboratory, such as rodents and flies, rely largely on olfactory cues to trigger aggression. This has limited our understanding of how other sensory cues are processed in the brain to evoke aggression. Here, I develop an innovative approach to study the circuitry underlying visually-evoked aggression in a novel model organism, *Betta splendens*. Betta have robust and highly stereotyped aggressive displays and visual input is enough to trigger them. I aim to determine the role that the amygdala in teleost fish, known as the dorsomedial telencephalon (Dm), plays in visually-evoked aggression. Like the mammalian amygdala, Dm receives visual information and modulates betta aggression. To determine whether Dm is necessary for visually-evoked aggression, I have developed tools to reliably evoke, quantify, and manipulate betta aggression. By using a supervised method of behavior segmentation, I have demarcated behavioral modules that define aggressive displays. To reliably evoke aggression, I have created a series of powerful animations of naturalistic and artificial stimuli that

pinpoint specific features of shape and motion that reliably trigger certain behavioral modules. Using a novel survival surgery stereotaxic procedure for betta, I am studying how the ablation of Dm impacts not only overall aggression, but visual attention and the predictability of specific visual features in evoking aggression. This work contributes to defining the precise role of Dm in betta aggression and provides a model for studying the neural circuitry underlying visually-evoked aggression.

F10 In sync for infants: Behavioral and hormonal signatures of care in biparental poison frogs

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Cooperation between parents ensures family success. Interparental communication is crucial for partnership stability, and occurs when mothers and fathers allocate distinct tasks to provide care to their offspring. However, little is known about the neural basis of parents' coordination of infant care despite its role in crucial decisions about infant feeding, neglect, or infanticide. One major challenge to successful parental coordination is how parents interpret offspring signals differently, but must synchronize their behavioral outputs to make important offspring care decisions. To understand how parents synchronize their behaviors in response to offspring signals, we studied the parental efforts of the biparental Mimetic Poison frog *Ranitomeya imitator.* First, we analyzed how moms and dads use acoustic communication to coordinate care of their tadpoles and tested which tadpole signals correlate to parental care effort. To do this, we used continuous monitoring of tadpole nurseries through cameras and acoustic recordings and measured levels of how often a parent visits tadpole sites and how often parental synchrony occurs. Our preliminary data suggests that males may use calls typically used for mating to mediate female behaviors, such as egg-provisioning for infants. Because steroid hormones can rapidly modulate social behaviors, we are currently quantifying circulating levels of steroid hormones of parents during varying stages of parenting, such as egg care, tadpole care, and tadpole transport, to determine which androgens are elevated or lowered at specific parental care tasks. Together, these data suggest that interparent synchronization may be mediated by hormone levels and acoustic signaling, and that parental responsiveness depends on infant signal.

F11 Combinatorial logic of neuromodulatory systems in the zebrafish telencephalon

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Animals must cope with an ever-changing environment, which requires them to adapt to a wide variety of challenges swiftly and appropriately. The nervous system is characterized by a plethora of modulatory systems such as neuropeptides and monoamines that change the way information is processed in the brain. Neuromodulation is thus one of the main factors enabling state-specific neural circuit function and the joint expression of several types of receptors in individual cell types might enable the combinatorial encoding of brain states. We investigated the differential expression of neuromodulators and their respective receptors in the zebrafish pallium and found that all major telencephalic areas could be classified with high confidence based solely on the expression of G-protein coupled receptors (GPCRs). In a more fine-grained analysis, we collected gene expression data from individual cells across the entire telencephalon and queried the extent to which neuronal cell types were embedded in different modulatory networks. Our data suggests that individual cell types commonly express more than one type of modulator and thus broadcast information along different modulator-receptor axes in addition to canonical synaptic signaling via neurotransmitters. Likewise, neurons are commonly characterized by the expression of several kinds of GPCRs. We hypothesize that concomitant activity of different neuromodulatory systems jointly encode brain states and enable the animal to appropriately act on sensory information.

F12 Sex steroids regulating year-round aggression: the role of neurosteroids across sex and seasons

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Sex steroids are important modulators of aggressive behavior. Although aggression may occur in many life stages, most research has been focused on aggressive behavior displayed during the breeding season, when circulating sex steroids are high. To further understand hormonal mechanisms underlying this behavior, animals that display aggressive behavior during the non-breeding season emerge as excellent models. Although non-breeding aggression shares common hormonal mechanisms in mammal and bird models, less research has been performed in other vertebrate groups, such as teleost fish. The weakly electric fish, *Gymnotus omarorum* is a territorial seasonal breeder that expresses year-round aggression in both sexes. Non-breeding aggression is expressed in castrated individuals and is modulated by estrogens. To further understand the role of sex steroids, we quantified by RT-qPCR the expression of 4 genes related to steroid hormones in 2 areas of the social brain network, from wild-caught breeding and non-breeding males and females (n=12 per group). We also quantified by LC-MS/MS 8 circulating steroids from the same individuals. Circulating estrogen levels were non-detectable during the non-breeding season in both sexes, while circulating androgens were present in both sexes. Even though circulating steroid levels were different across seasons, we found no significant seasonal difference in the gene expression of aromatase, androgen receptor, α and β estrogen receptors in the social brain of both sexes. We propose that circulating androgens can act as precursors for estrogen neurosynthesis and that the brain-derived estrogens can modulate the expression of aggressive behavior in different physiological conditions, independently of circulating hormone levels.

F13 Understanding the control of mouthbrooding behaviour in the African cichlid *Astatotilapia burtoni*

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Parental care behaviours can take varied forms, with mouthbrooding being one of the most extreme examples. Mouthbrooding is characterised by retrieving embryos into the mouth to be held during development and is common among cichlid fishes. This project aims to dissect the neural mechanisms underlying mouthbrooding using the African cichlid *Astatotilapia. 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 this 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 the expression of *cFos* (a marker for recent neural activity) and candidate receptors and neurotransmitters. (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 with a sparser activation in lateral regions of the VL. Additionally, we find that mothers are more likely to re-initiate mouthbrooding if the brood has hatched and that brood retrieval times decrease with advancing developmental stage. We discuss how these results will enable the dissection of which neuronal populations and circuitry may be responsible for mouthbrooding.

F14 Differences in brain activation patters between populations artificially selected for sociality in zebrafish (*Danio rerio*)

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Sociability, or the capability to interact with other members of one's species, is a critical ability to survival and reproduction. In vertebrates, this faculty is regulated by a set of organized brain nuclei denominated social decision-making network (SDMN). But there are species and individuals more social than others and the current debate is whether or to what extent the underlying neural mechanisms that control social behaviours differ among them. To answer this question, we have been doing an artificial selection experiment with zebrafish (Danio rerio). We phenotyped a base (F0) population of Tuebingen (TU) zebrafish for sociality using a social preference test. The test consisted in presenting a social stimulus (a video-playback of a shoal of four fish) against a non-social stimulus (a video-playback of a "shoal" of four objects, circles, moving randomly in the same background). We established four F1 selection lines from the F0 by crossing fish with the highest (Shoal preference line), the lowest (Circles preference line), equal (No-preference line), or random (Random preference line) preference for shoal. The selection experiment has reached the F5 generation with a clear increase in shoal preference of the shoal preference line compared to the other three lines. We have now characterized brain activation patterns with pS6 (the phosphorylation of P6 ribosomal protein) immunofluorescence in response to social vs. non-social stimuli in sub-samples of fish from each line. Our goal is to describe differences in the molecular identity of functional populations of neurons between the four artificially selected populations, within the SDMN. We will present the preliminary results of these tests in two of the selected lines.

F15 Social communication signals in synodontid catfish, social preferences and neural correlates

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Synodontid catfishes generate swim bladder vibrations for sound production, which are established social communication signals in fishes. In some synodontid species the sound producing muscles have been converted into an electric organ, thus changing the communication signal modality. Synodontids thus offer a unique opportunity to study the transition of one social communication system to another in closely related species.
As conspecifics recognition has not yet been investigated in Synodontis catfish, we first conducted a series of behavioral experiments to demonstrate species specific preference and to determine which sensory components mediate this cognitive ability in three synodontid species using either sound, electric or both signal types (Synodontis grandiops, S. nigriventris and S. eupterus). We performed a series of preference tests and found that syndontids prefer to associate to their own species than to heterospecifics, thus showing that synodontids can recognize conspecifics. As species-specific social recognition is mediated by a range of sensory cues in other animals, we are currently investigating the preference of either species to different sensory components (electric or sound) by playing back different types of social communication signals. To understand how the social behavior network adapted to such a change in communication signal (acoustic to electric), we are investigating the neural correlates of social signal perception using PS6 expression, a correlate of neuronal activity.

F16 Context-dependency of social affiliation in zebrafish

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↑UP

Many animals live in groups and affiliate with conspecifics. Social affiliation allows individual animals to access shared information on prey and mates, and dilutes the risk of predation. It has long been noted that the expression of social behavior depends on environmental context and internal state. To investigate modulatory influences on the processing of social information, we analyze shoaling behavior of groups of zebrafish in the context of threat perception and stress. To that end we examine *grs357* mutant zebrafish, which lack functional glucocorticoid receptors (GRs) and have a chronically elevated stress axis. We hypothesize that the shoaling behavior of these mutants is altered. We also examine wildtype zebrafish exposed to alarm pheromone (AP, a. k. a. 'Schreckstoff'), a component in the skin of fish that is released upon injury and triggers fear responses. AP activates olfactory glomeruli with projections to the habenula, a brain region modulating fear responses. AP has been reported to increase aggregation in adult zebrafish. However, it is unknown whether AP affects zebrafish social behavior at early developmental stages, in which whole-brain functional imaging is more readily feasible. Therefore, we will investigate the developmental stage at which threat perception and stress first influence social behavior using computer vision techniques and quantitative behavioral analysis, with the goal to identify a phenotype whose neural circuit function can be monitored at a later stage in the project. This study will elucidate neural mechanisms of context-dependent modulations of social affiliation.

F17 South American annual fish *Austrolebias reicherti* increase motivation to court and fight as lifespan elapses. Differential responses to stress across the season?

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Glucocorticoids (GC) are key hormones in the adjustment of social and reproductive behavior to the biotic and abiotic environment. However, these interactions have been mainly addressed in species with traditional stress-reproduction relations. Annual fish of the genus *Austrolebias* inhabit temporary ponds that dry out during summer. Adults breed continuously during a single season characterized by the impoverishment of environmental conditions. Males engage in intense fighting and actively court and defend territories with females. In males, circulating GC levels increase as season develops and GC promote courtship performance in captivity. Our goal was to characterize agonistic and sexual behavior and assess the role of GC at two stages of the season: early (ES) and late season (LS). Adult *A. reicherti* males and females were collected from natural habitat, transferred to laboratory facilities and housed individually. N_{ES}=19 and N_{LS}=21 males were subjected to a 7-days treatment with 150 ng/ml of hydrocortisone (GC) or vehicle after which sexual and agonistic behavior were sequentially tested for 20 min each. Males increased both their motivation to court and fight towards LS, as they reduced their latency to initiate courtship, performed more courtship displays and reduced the latency to attack an intruder. GC effect was not statistically significant at either stage of the season. This study shows an increase in reproductive investment both in terms of male competition and courtship behavior as lifespan elapses and suggests a behavioral tolerance to stress at the beginning of the season. Further experiments should address whether the seasonal increment in reproduction investment is related to the rise in GC in these particular teleost with such an extreme life cycle.

F18 Dark/light preference as a measure of stress and anxiety in fighter and wild-type strains of the siamese fighting fish *Betta splendens*

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Siamese fighting fish *Betta splendens* have been selected over centuries for paired-staged fights, where matched for size males fight in a small tank. Previous studies showed that this artificial selection for winning impacted the hypothalamus-pituitary-interrenal (HPI) axis, with fighter fish having lower circulating cortisol levels and being less affected by stressors, as compared to wild-type. This difference makes *B. splendens* an interesting model to study the genetic and physiological mechanisms of stress/anxiety in fish. Scototaxis (dark/light preference) test explores the natural preference of species for dark or light areas and it is one of the most commonly used assays to measure stress/anxiety in animals, as stress/anxiety increases the time spent in the preferred area. Here, we compared the dark/light preference of different *B. splendens* strains as larvae and as adults. Our results show that *B. splendens* develop a strong preference for dark as soon as 4 days post hatching (dph). However, this preference was similar in larvae from fighter, wild-type and also other domesticated strains. Adult fish consistently showed a preference for the dark areas but here results suggest possible differences between strains. These results indicate that the reported differences between wild-type and fighter strains in HPI activation develop after at least one-month post hatching. The study further provides suggestions for the standardization of the assay in larvae and adult *B. splendens*.

CHEMICAL SENSING I

G1 Olfactory gating of visual preferences in *Aedes aegypti*

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How mosquitoes locate their host and determine which individuals to bite has important consequences for human health. Aedes aegypti, the yellow-fever mosquito, is known to use multiple cues, such as temperature and odors, to locate its host. However, little is known about the visible spectra that attract mosquitoes and how odors such as CO2 can modulate their visual search. 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 CO2 induces a strong attraction to long wavelengths (orange and red) but does not trigger behavioral change toward green and blue wavelengths. Interestingly, long wavelengths dominate the human skin color spectrum, and filtering those wavelengths abolished the observed attraction. We then tested different mosquito species and found that preferences are species-specific, likely due to different ecological niches. Finally, two-photon excitation microscopy in tethered mosquitoes confirmed that activity in the lobula, an optic lobe of the mosquito brain, is modulated by olfactory processing of CO2. Together, our results unraveled novel aspects of host-seeking behaviors and have the potential to open new avenues for controlling attraction to human hosts.

G2 Functional and developmental analyses of the sex pheromone reception system in the American cockroach during the nymphal-adult transition

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Adult male cockroaches *Periplaneta americana* are attracted to female-produced sex pheromones, periplanone-A (PA) and -B (PB), and exhibit courtship rituals in the close proximity. On the other hand, nymphal males exhibit neither orientation nor courtship behaviours toward the sex pheromones. Thus, behavioural responses to the sex pheromone are altered during the nymphal-adult transition. In adult males, PA and PB are specifically received by PA- and PB-responsive sensory neurons (PA-SNs and PB-SNs), respectively, in male specific *single-walled* B (*sw*-B) sensilla. In the nymphs, we found that PB is received by the PB-SNs in a different type of sensilla, termed the *sw*-A2 sensilla, which is characterised by a shorter shaft length compared with adult *sw*-B sensilla (Tateishi et al., 2020). In this study, we confirmed that nymphal *sw*-A2 sensilla transformed into adult *sw*-B sensilla by the elongation of their shaft at the final moult, and the PB response sensitivities of PB-SNs increased with the sensillar elongation. In addition, using systemic RNAi combined with single sensillum recording (See Tateishi et al., 2022 for details), we functionally characterised PA and PB receptors expressed in the cockroach antennae for the first time. Furthermore, by comparing the expression of PB receptor gene between nymphal and adult antennae, we partially revealed the molecular basis of the enhancement of the sex pheromone reception during the nymphal-adult transition. These results strongly suggested that the developments of physiological properties of sex pheromone-responsive SNs during nymphal-adult transition play a critical role in the expression of sexual behaviours.

G3 Ants act as olfactory bio-detectors of tumour in patient-derived xenograft mice

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Early detection of cancer is critical in medical sciences, as the sooner a cancer is diagnosed, the higher the chances of recovery. Tumor cells are characterized by specific volatile organic compounds (VOCs) that can be used as biomarkers to detect cancer. Through olfactory associative learning, dogs or mice can be trained to detect these VOCs and act as a living detection tool. Insects, such as ants, have a refined sense of smell and can be easily and quickly trained using olfactory conditioning. Ants are known for their reliable olfactory memory and precise discrimination abilities. They could therefore represent ideal bio-detectors. Using urine from patient-derived xenograft (PDX) mice as stimulus, we demonstrate that individual ants of the species *Formica fusca* can be trained to discriminate the odor of healthy mice from that of mice bearing a tumor, and do so after only three conditioning trials. Chemical analyses confirmed that the presence of the tumor changed the urine odor of PDX mice, supporting the results of the behavioral experiments. Our study demonstrates that ants reliably detect tumour cues in mice urine and have the potential to act as efficient and inexpensive bio detectors for cancer.

G4 Neurophysiological correlates of alcohol tolerance in the mushroom body of *Drosophila melanogaster*

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In nature, the fruit fly, *Drosophila melanogaster,* feeds on decaying fruits containing varying concentrations of alcohol —a by-product of fermentation. Consumption of low concentrations of alcohol can result in increased fitness, whereas higher concentrations can cause significant developmental delays and cognitive dysfunction. Thus, it is not surprising that adult flies can adapt to the presence of alcohol in food and minimize some of its detrimental effects. However, the neural mechanisms by which these adaptations occur remain unresolved. Here, we use a Drosophila model to explore the neurophysiological correlates of alcohol tolerance in the Mushroom Body of the fly—a key neuropil in the brain that integrates sensory signals. Using a functional imaging approach, we studied the neurophysiology of the MB calyx in response to ethanol. Flies expressing the calcium sensor protein GCamp6s in MB neurons were treated with ethanol vapor (~95%) until anesthetized (~40 minutes). Twenty-four hours later, we recorded the intracellular calcium dynamics in the MB calyx. Adapted flies showed a decreased response to a short ethanol exposure (3 seconds) compared to control flies, suggesting that this brain area is involved in the neurophysiological processing of ethanol tolerance. The reduction in neural signaling correlates with the changes observed in *Drosophila* behaviors related to ethanol exposure, suggesting a possible neural substrate for the behavioral adaptation to alcohol during tolerance.

G5 Two metres as the mosquito flies – The role of a select odorant receptor regulating the onset of host seeking in the malaria mosquito *Anopheles gambiae*

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Female anthropophilic mosquitoes, such as the malaria mosquito, *Anopheles gambiae*, show a strong preference for human hosts – a behaviour that is predominantly driven by olfactory cues. However, this attraction to human host cues only develops during adult maturation. Mature females are attracted by human odour, whereas attraction in newly-emerged females is inhibited. This onset of the host-seeking behaviour is correlated with age-dependent changes in odorant receptor (OR) gene transcript abundance, among which *AgamOR39* is a promising candidate driving the behavioural change. Here, we show that female *An. gambiae* lacking a functional OR39 exhibit an abolished physiological response to its ligand, sulcatone, a compound enriched in human body odour. Assessing the behaviour of mutant mosquitoes in a wind tunnel assay is currently ongoing. Results will contribute to understand the molecular mechanism regulating the onset of host seeking in *An. gambiae*, a behaviour resulting in a large proportion of the world's population being at risk of infection by mosquito-borne diseases.

G6 Optimized functional imaging of mosquito olfactory sensory neuron activity

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Mosquitoes are responsible for transmitting deadly and debilitating diseases such as malaria, dengue and Zika. The yellow fever mosquito *Aedes aegypti* and the African malaria mosquito *Anopheles gambiae* are highly anthropophilic and exhibit strong innate sensory drives to seek out humans. Both mosquito species detect multiple physical and chemical cues emanating from the human body to locate a host to blood feed on. Amongst these cues, human odor is crucial for long range host detection and is sensed by specialized olfactory sensory neurons in sensilla that are primarily localized to the antennae and maxillary palps. These neurons can be divided into different classes based on their expression profile of chemoreceptor genes. To date, heterologous expression systems and electrophysiological approaches such as single sensillum recordings and electroantennography have facilitated studies of the ligand tuning dynamics of different OSN populations and target chemoreceptors from these disease vectors. To increase the throughput of mosquito neurophysiological studies and comprehensively decode the ligand specificity and sensitivity of OSN populations in *Ae. aegypti* and *An. gambiae*, we applied CRISPR-Cas9 T2A-In Frame Fusions and the QF2-QUAS system to gain genetic access to broad OSN subsets in both species, including those expressing the olfactory co-receptors Gr1, IR8a, IR25, IR76b and Orco. We have used these reagents in conjunction with QUAS-GCaMP responder lines to visualize peripheral and central responses to CO_2 and other ligands present in human scent. These optimized methods for imaging mosquito neural activity have the potential to be applied to study the molecular and cellular basis of mosquito attraction to humans and other facets of their sensory biology.

G7 Honey bees' olfactory discriminative abilities rely on a community of gut bacteria

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Following the recent discovery that gut bacteria could influence their host's behaviour, the study of the microbiota gut-brain axis has emerged as a new field of research. Most research has focused on humans, which are not experimentally amenable, and laboratory animal models (e.g. rodents, fruit flies), whose gut microbial community and environment lack ecological complexity. To propose the Honey bee as a new model to study the microbiota gut-brain axis, we started by asking whether the gut microbiota could influence honey bees' learning capacities. The gut of newborn bees is naturally microbiota depleted. It was artificially colonized by the five core bacterial phylotypes either in association (conventionally colonized bees), or alone (monocolonized bees). Using the well-established olfactory conditioning of the proboscis extension response, we assessed the ability of gnotobiotic bees to learn the association between an odour and a sucrose reward while another odour was left unrewarded. We show that conventionally colonized bees were better able to discriminate the two odours compared to microbiota-depleted and monocolonized bees. This is the first demonstration that the community of core gut bacteria contribute to the sophisticated olfactory discriminative abilities of honey bees. It paves the way for future research on the neurobiological mechanisms underlying this effect.

G8 Neuronal processing of trail pheromone communication in the ant *Crematogaster scutellaris*

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The sense of smell is the most prominent sensory modality for ants. In these social insects, chemical communication is essential for colony organization and survival. For example, trail-following behavior, alertness, recruitment, or signaling of the reproductive state are coordinated by the action of pheromones. Besides these signals, other environmental odors are important for orientation and evaluation of food sources. Thus, the detection, processing, and recognition of a large number of chemical cues by the olfactory system have a crucial role in the survival and reproductive success of ant colonies. With the main goal of understanding whether ants express specific neural circuits for processing social and non-social odors, here we present a neuroethological study of trail-pheromone processing in the Mediterranean acrobat ant *Crematogaster scutellaris*. First, a neuroanatomical characterization of the antennal lobes revealed the presence of a macroglomerulus, a structure that is believed to be involved in trail pheromone processing in other ant species. Second, an analysis of the trail pheromone, whose source is the tibial gland, showed that its chemical composition may be similar but not identical to the one previously determined for the congeneric species *Crematogaster castanea*. Third, by a quantitative behavioral study we identified single chemical compunds possibly invoved at different degrees in the trail-following communication system of *C. scutellaris*. Finally, preliminary results from two-photon calcium imaging experiments suggest that the trail pheromone is processed in specific glomeruli, segregated from those processing non-social plant-derived odours, thus providing a direct evidence of a specialization in the brain for trail pheromone processing.

G9 Alarm! - modulatory effects of Schreckstoff on the startle escape response of goldfish (*Carassius auratus*)

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In many fish, two large reticulospinal neurons, the Mauthner cells (M-cells) integrate multimodal sensory inputs, and a single action potential in one M-cell is sufficient to activate contralateral motor networks for a powerful and fast (<12ms) startle response (C-start). The one-to-one relationship between action potential and startle allows us to assess M-cell excitability by measuring startle probability and latency. Like in many species, startle behavior in fish shows remarkable plasticity in response to changes in the environment and/or internal physiological states. One powerful modulator of startle is prepulse inhibition (PPI), a sensory gating process that largely reduces the expression of startle behavior. PPI however, is disrupted in African cichlid fish exposed to a chronic social stressor (bullying). Here we asked how an acute stressor namely, an alarm pheromone (Schreckstoff) released from an injured fish will affect the startle response/PPI and swimming behavior in goldfish. The result showed a significant reduction (mean 10.6% +/- 5.5% SEM) in startle latency ($p=0.045$; GEE) after the application of the alarm pheromone however, startle probability and PPI were not affected. We also found that exposure to the alarm pheromone significantly decreased distance traveled, average velocity, and thigmotaxis ($p<0.0001$ for all three parameters; GEE) in the tested fish. In conclusion, the fact that the alarm substance reduced response time without changing startle probability (i.e., M-cell excitability) may indicate an effect of the drug on the presynaptic auditory pathway. The latter notion will be tested in ongoing electrophysiology experiments.

↑UP

MECHANOSENSATION, ANEMOSENSATION, THERMORECEPTION, HYGRORECEPTION AND NOCICEPTION

H1 Uncovering the molecular mechanisms of rapid experience-dependent thermosensory adaptation in *C. elegans*

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The nematode *C. elegans* compares the ambient temperature with its prior temperature experiences in order to move towards a preferred temperature on a thermal gradient in a well-studied behavior called thermotaxis. Thermosensation is mediated by thermosensitive guanylyl cyclases expressed in the single AFD neuron pair, the primary thermosensory neurons in *C. elegans*. The temperature threshold at which AFD responds is determined by prior temperature experience, and when animals experience a new warmer temperature, the thermosensory response threshold of AFD adapts on a rapid minutes-long and a slower hours-long timescale. The rapid adaptation allows AFD to remain responsive to local temperature variations, whereas the slower adaptation of both AFD sensory responses and synaptic output allows the animals to migrate towards its preferred temperature. Although transcriptional mechanisms underlie long-term temperature adaptation of AFD responses, the mechanisms regulating rapid adaptation were unknown. By expressing cGMP-sensitive fluorescent sensors in the AFD neurons, I determined that cGMP dynamics in AFD also adapt rapidly, and that this adaptation is calcium-dependent. Response plasticity is partially mediated by the neuronal calcium sensor NCS-2, as well as the cGMP-dependent protein kinases EGL-4 and PKG-2. Disrupting phosphodiesterase activity or mutating phosphorylation sites on the thermosensory guanylyl cyclases also results in perturbed short-term adaptation. My current results suggest that rapid adaptation is a function of intracellular feedback mechanisms initiated by temperature-regulated increases in intracellular calcium and cGMP levels. Ongoing experiments aim to identify further targets in AFD that mediate rapid thermosensory adaptation.

H2 Characterization of the humidity receptor neurons in *Drosophila melanogaster*

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Humidity is an omnipresent environmental factor influencing fitness, reproductive behaviour and geographic distribution of terrestrial animals. Insects are particularly sensitive to humidity levels and common disease-vectors like the malaria mosquito (*Anopheles gambiae*) and the tsetse fly (*Glossina pallipides*) rely on humidity cues to find their host and egg-laying sites. Even though humidity sensing is crucial for a wide range of animals, the underlying neuronal basis remains poorly understood.

Specific neurons for humidity sensing, the hygrosensory receptor neurons (HRNs), have been described and studied in a wide variety of insects. In *D. melanogaster* the HRNs are located within sensory sensilla in two out of three separate chambers in the sacculus, an invagination in the posterior side of the antenna. Each sensilla houses a triad of neuronal cells: one moist, one hygrocool and one dry neuron. The sensilla of the two sacculus chambers have unique morphology and the HRNs in the separate chambers project their axons to different glomeruli in the antennal lobe of the brain. The current study focusses on investigating if this anatomical heterogeneity is matched by a molecular heterogeneity, using single-nucleus RNA sequencing. This technique allows for the classification the different neurons based on their transcriptomic profiles.

In a cluster analysis the different types of HRNs in the two sacculus chambers seperate into clearly distinguishable groups. Based on the individual expression profiles of these groups we have identified a range of candidate genes. Their involvement in mediating hygrosensation is assessed in a behavioural assay. The results of this study will bring us closer to understanding the neuronal basis of humidity sensing behaviour.

H3 Cephalic mechanosensors and their role in initiation of flight related reflexes

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During flight, insects sense air movement via sensory bristles distributed all over their body. These bristles provide mechanosensory feedback to their nervous system and modulate wing motion to cope with ambient wind conditions. In many insects including locusts, moths, etc., flight can be rapidly initiated by stimulating a set of bristles called cephalic hair on their head (Camhi, 1969). Rapid initiation requires diverse, distributed flight-related reflexes to be simultaneously activated. Thus, we hypothesized that cephalic hair directly and simultaneously trigger diverse flight-related reflexes. We tested this hypothesis using Oleander hawkmoth, *Daphnis nerii*. Our experiments revealed diverse facets of cephalic hair-mediated flight initiation in tethered hawkmoths. First, at the behavioral level, we observed that moths stimulated with an air puff directed at their head, displayed a robust, rapid and stereotypical flight initiation response and became hyperactive. Such flight initiation activated multiple reflexes including leg extension, wing initiation, antennal positioning, head stabilization, and abdominal flexion. Large moths like Daphnis nerii, require flight muscles to be warmed through a prolonged low amplitude wing motion during voluntary flight. However, thermograms of thorax of moths showed that this warm-up phase was bypassed following cephalic hair stimulation. Primary neurons of these hairs project from brain into the metathoracic ganglion, arborizing in the region of flight motor neurons. Electromyograms of the steering muscles innervated by flight motor neurons showed consistent activity upon stimulation of cephalic hair. Thus air puff activation causes cephalic hair afferents to activate flight through serial activation of flight-related reflexes.

H4 Characterization of a leg mechanosensor in the Oleander hawkmoth

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The physical stimuli sensed by mechanosensory organs are processed in two ways. First, the cues are filtered by the mechanics of the structures of the sensory organs. Second, the mechanically-filtered cue is encoded by neurons with specific tuning properties that filter the sensory signal. Although much effort has gone into the understanding of the neural filtering of sensory signals, we know relatively little about how mechanical pre-filtering influences a sensory cue. To address this, we investigated the structure of a proprioceptor called the femoral chordotonal organ (FeCO), in insect legs. FeCO senses the movements of tibia relative to femur via a complex sensory apparatus that converts the rotational motion of tibia into the axial stretch and relaxation of the organ. As a first step, we characterized the anatomy and morphology of FeCO in the Oleander hawkmoth *Daphnis nerii* using diverse techniques, including microCT and confocal microscopy. In *D. nerii*, FeCO is located proximally within the femur, and consists of two conically-shaped subunits. These subunits are mechanically coupled to tibia via a rigid rod-like structure, called an apodeme. Vastly different in their size, the two subunits contain 8 and ~88 neurons respectively. Previous studies on locusts suggest that the subunits are functionally distinct, such that one senses vibrations and the other senses slow joint movements. Because hawkmoths use their legs in functional contexts that are very different from locusts, we aim to understand how the subunits are functionally organized in moths, and how the mechanical signals presented to the leg are transduced by the structure of the FeCO. Together, these data will enable a better understanding of the mechanical prefiltering by the structure of the FeCO.

H5 Suppression of host nocifensive behavior by parasitoid wasp venom

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The jewel wasp *Ampulex compressa* envenomates the brain of its host the American cockroach *(Periplaneta americana)*, making it a behaviorally compliant food supply for its offspring. The main target of the wasp sting is a sensorimotor command center in the brain, known as the "central complex" (CX). The wasp venom induces a behavioral sequence in the host beginning with an intense bout of grooming (~20–30 min), followed by the onset of a long-lasting (5–7 days) sedentary condition termed “hypokinesia.” During this hypokinetic state, stung cockroaches do not respond to several harmful stimuli. E.g., the wasp breaks off both antennae to drink hemolymph from the stumps. The wasp also pulls strongly on one of the antennae while dragging the host to the nest. Given these observations, we hypothesize that the CX has a role in processing noxious input and that the wasp venom modulates the CX activity to suppress nocifensive behavior. To investigate this, we examine how envenomation compromises nociceptive signaling pathways in the host. Noxious stimuli applied to the cuticle of stung cockroaches fail to evoke escape responses, even though nociceptive interneurons projecting to the brain respond normally. Hence, while nociceptive signals are carried forward to the brain, they fail to trigger robust nocifensive behavior. In contrast, we found that noxious stimuli evoke weaker responses in the CX after venom injection. Total evoked activity, evoked firing rate, and duration of noxious-evoked activity in this brain center decrease, causing the desensitization of nociceptive response. Our findings demonstrate how the reproductive strategy of a parasitoid wasp is served by venom-mediated elimination of aversive, nocifensive behavior in its host.

H6 The antennae as wind detectors during straight-line orientation in dung beetles

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Upon arrival at a dung pile, the South African, ball-rolling dung beetle *Kheper lamarcki* is met with fierce competition for food. To escape this, the beetle quickly constructs a ball of dung and departs with it from the pile along a straight path (Byrne et al., 2003). To consistently move along a set bearing is not trivial, and studies have shown that dung beetles rely on the directional information given by celestial cues to steer straight (Dacke et al., 2011, Dacke et al., 2013a, Dacke et al., 2014, el Jundi et al., 2014). Beetles also possess a wind compass (Dacke et al., 2019), however, few studies have explored precisely how directional information is extracted from prevailing wind. Work on other insect species suggest that the Johnston’s organ (JO), present in the pedicel of the antennae, is involved in wind perception (Linsenmair, 1972, Boo and Richards, 1975, Hallberg, 1981). To characterise the possible role of the antennae in extraction of directional information from wind to support straight-line orientation, we combined behavioural experiments with morphological analyses. Histological sections of beetle pedicel revealed the presence of the JO, comprised of roughly 104 sensory units radially arranged within this antennal segment. The behavioural trials showed that beetles with intact antennae were able to perform anemomenotaxis, while individuals that lacked one or both antennae failed to consistently move along a given bearing. It should be noted that these beetles were able to orient when presented with a simulated sun cue, showing that the antennal manipulations did not impair the motivation to perform accurate straight-line orientation. Taken together, these results suggest that it is, indeed, the antennae that contain the wind sensor of *K. lamarcki*.

H7 Active anemosensing: How insects could estimate wind direction through sensory integration

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Estimating the direction of ambient fluid flow is a key component of plume tracking behaviors for flying and swimming animals. How animals accomplish this remains unknown. In this work I present a unifying mathematical framework to explain how previously described behaviors and sensory encodings found in the fruit flies’ brain can together make it feasible for them to continuously estimate the direction of ambient wind. Recent calcium imaging experiments with tethered flying Drosophila have shown that flies encode the angular direction of multiple sensory modalities in their central complex: orientation, apparent wind (or airspeed) direction, and direction of motion. Together these angular measurements are sufficient to estimate wind direction, but require a computationally intensive iterative optimization scheme. Flies exhibit visual motor reflexes to maintain relatively constant ground speed, a behavior which can dramatically simplify the required calculations. Using a series of simulations I show that even with this simplification there are two fundamental requirements for continuously estimating ambient wind direction from angular sensory modalities. First, it is necessary to estimate some form of time derivative of at least one of the sensory modalities. Second, continuous changes in direction of motion are required and information must be integrated across these changes in direction for the computations to be well posed. Together, the mathematical framework and simulations provide a theory for what types of computations we should expect to find the flies brain and also suggest that ambient flow estimation may be an important driver underlying the zigzagging maneuvers characteristic of many plume tracking animals' trajectories.

VISION AND PHOTORECEPTION I

I1 Functional evidence of the role of the crab lobula plate as optic flow processing center

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Rotational motion produces a wide drift of the visual panorama over the retina of animals, termed optic flow (OF). Such motion is stabilized by compensatory behaviors (driven by the movement of the eyes, heads or the whole body depending on the animal) collectively termed optomotor response (OR). It has long been known that, in the visual system of flies, the lobula plate is the center involved in OF analysis and in guiding OR. Recently, a crustacean lobula plate was characterized by neuroanatomical techniques in the mud crab *Neohelice granulata*, sharing many canonical features with the dipteran neuropil. This lead to questioning if a common functional role is also shared. In this work we tackle that question by performing electrolytic lesions followed by behavioral testing. Results show that crabs with lesioned lobula plates fail to execute OR (or present a poor and unsynchronized response) in comparison to both control-lesioned (presenting a lesion of similar size but in another region of the optic neuropils) and non-lesioned animals. The lesion of the lobula plate cause a specific impairment in the OR, since avoidance responses to an approaching visual stimulus were not affected. These results present strong evidence supporting that a similar neuroanatomical structure in crabs and flies, the lobula plate, carries out the same function.

I2 Population coding of visual information and control of avoidance behaviours in locusts

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Locusts are capable of performing complicated flying maneuvers, which relies on rapid detection of moving objects present in the visual field to generate appropriate behavioural responses. An identified neural pathway, comprised of the Lobula Giant Motion Detector (LGMD) and the Descending Contralateral Motion Detector (DCMD), responds preferentially to approaching objects. LGMD receives retinotopic inputs from ipsilateral ommatidia and generates spikes in a 1:1 ratio in the DCMD, which synapses with multiple locomotion-related neurons. As an angular threshold detector, the DCMD has been implicated as critical for initiating evasive behaviours, although its specific role remains to be fully described. Importantly, numerous other motion-sensitive neurons have also been identified in locusts. These neurons have distinct firing properties and response preferences. Information from these neurons likely contributes to production of avoidance behaviour. However, few studies have investigated the contribution of these neurons on a neural population level. To better understand how visual information is perceived by locusts, we constructed a multichannel recording system within an existing flight simulator and presented various complex visual stimuli to rigidly tethered locusts. Preliminary analyses have identified functional units that responded to aspects of visual motion. Common trends, which reflect the activity of neural ensembles, were extracted from these functional units. These common trends were compared under different conditions to reveal how population coding is modulated dynamically.

↑UP

I3 Investigating the synaptic connections in the lamina of the praying mantis *Tenodera sinensis*

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The first neuropile of the optic lobe of insect brains, the lamina, consists of thousands of functional units, the cartridges. A cartridge corresponds to a single ommatidium and has a defined set of neuronal elements, fibres of the photoreceptors and neurites of monopolar cells. To understand the processing of visual information in the lamina, it is important to know the synaptic connections between photoreceptor cells and downstream monopolar cells, which are well documented in animals such as flies, butterflies, or dragonflies. However, for the praying mantis, there is only spares information available. Some information is given by Kral and Prete (2004) on the cell types which can be found in the cartridges: 3 large monopolar cells and 2 or 3 small monopolar cells, but there is no thoroughly study looking at the connectivity between these cells and also between the cartridges. The praying mantis is a sit-and-wait predator and does not depend on flying as much as the species mentioned before. Therefore, we are interested if there are any significant differences in the connectivity pattern of the neuronal elements within and between cartridges in the lamina. Here we present the first results from the praying mantis *Tenodera sinensis* (L3 larvae) using the electron microscopy technique ATUMtome SEM to trace neurons within the lamina and document the synaptic connections. We can show that there are synaptic connections formed between different neurons (large and small monopolar cells) not only within their respective cartridge but also to neighbouring ones and cartridges from one or two cartridge-rows away.

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I4 The spectral organization of the retina and lamina of the butterfly, *Papilio xuthus*, with the animals' best color vision

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The compound eyes of the butterfly *Papilio xuthus* contain ultraviolet (UV), violet (V), blue (B), green (G), red (R), and broadband (BB) photoreceptors (PRs). Each ommatidium bears nine PRs, R1-R9, in one of three fixed combinations (UV/B/G/R, V/G/BB, or B/G), making the eye a collection of three spectrally distinct ommatidia types. The nine PRs project axons to the lamina, where they form a cartridge with four or five second-order lamina monopolar cells (LMCs). In a connectome analysis [1)], we identified five LMC types, L1-L5, according to their arborizations in the lamina and medulla. We also analyzed the synaptic connections among PRs and LMCs in the lamina and identified 54% of the synapses were from PRs to LMCs. Interestingly, 25% of synapses are between PRs (inter-PR), absent in the *Drosophila* lamina. Inter-LMC synapses (8%) and feedback from LMCs to PRs (12%) also exist. 76% of inter-PR connections are between spectrally different PRs, which contribute to creating spectral-opponent PRs. The LMCs receive inputs from all PRs within their "home" cartridges with slightly variable weights. As a result, the LMCs exhibit broad spectral sensitivity whose profiles depend primarily on the ommatidia type but not necessarily on the LMC type. The spectrally complex visual system eventually creates the cortical-like color-encoding neurons in the mushroom body [2)], which potentially provide the physiological basis of the *Papilio xuthus*' accurate wavelength discrimination that outperforms humans [3)].

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I5 Mixes and matches of visual pigments: fascinating innovations of the snake visual system

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Snakes (ca. 4,000 extant species) are exceptionally diverse, and so are their retinas and visual cells. During their evolution from lizards, snakes lost several visual structures, such as ciliary muscles, cone oil droplets, and two visual opsins, likely as adaptations to low light conditions. Within Caenophidia ("advanced" snakes), new evolutionary trajectories promoted innovative visual system adaptations, including unique double cones, cone microdroplets, and transmuted photoreceptors (cone-like rods, rod-like cones). Snakes have up to three visual pigments, with a short- (SWS1) and medium/long-wavelength (LWS) opsin, and rod-rhodopsin (RH1). In non-caenophidians, LWS and SWS1 opsins are expressed in distinct cones and RH1 in rods. In diurnal caenophidians, simplex all-cone retinas occur, with RH1 expressed in cone-like photoreceptors, in addition to SWS1 and LWS. We present new data for immunolabelled retinal sections and wholemounts of dipsadid caenophidians. These identify distinct SWS1 and LWS cones and RH1 rods in nocturnal taxa. In diurnal dipsadids, RH1 and SWS1 are co-expressed in cones to varying degrees. RH1 is also co-opted by a small population of LWS cones. Photoreceptors co-expressing SWS1 and RH1 are widely distributed within retinas, but exclusively RH1 and exclusively SWS1 photoreceptors are spatially restricted. We consider it plausible that the pure RH1 and SWS1 photoreceptors might be involved in chromatic pathways, along with LWS, while cones co-expressing both photopigments might be devoted to contrast and luminance pathways. Visual opsin co-expression was not previously known for any caenophidian, and co-expression of the rhodopsin with cone opsins, as far as we are aware, seems to be unprecedented for vertebrates.

I6 How does the common barbel (*Barbus barbus*) see? The effect of the whole-genome duplication on vision

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Teleosts outstand among vertebrates in the number of visual pigments and photoreceptor types. Such extraordinary repertoire has evolved also thanks to the whole-genome duplication that occurred in their ancestor, approximately 350 million years ago. Here we explore teleost fish species that has recently experienced subsequent whole-genome duplication - the common barbel (*Barbus barbus*). We focus on the effect of tetraploidy on the visual system. We found 13 opsin genes in the common barbel genome – an unusually high number. We further investigate opsin gene expression to test if multiple opsin genes in the genome result in different visual system function. We present opsin expression profiles of adult specimens and larvae at different developmental stages and we have identified ontogenetic shifts specific for barbel, as well as shared among teleost fishes. Both copies of the opsin genes resulting from the barbel-specific whole-genome duplication are expressed in retina, and for some opsins (SWS1, SWS2, RH2) we found alternative gene expression of the two copies during development. We also visualise opsin expression in adult retinae via fluorescence in situ hybridization to reveal the retinal cone mosaic and we discuss its functional consequences.

I7 A model of a locust looming detection circuit incorporating global, lateral and feedforward inhibition

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Detection of looming obstacles is vitally important to all animals for avoiding predators, conspecifics, and environmental obstacles. The migratory locust, *Locusta migratoria*, possesses a well-characterized neuron in each optic lobe known as the lobula giant movement detector (LGMD) which integrates visual data into a signal encoding the imminence of collision with an approaching object. While this neuron itself and certain portions of its input network are well-studied from both physiological and modelling perspectives, certain discoveries in recent literature have not yet been widely reflected in computation models of locust looming detection. Specifically, the posited role of global inhibition in normalizing inputs to the LGMD has only recently

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been investigated computationally; moreover, detailed characterization of the number, behavior and receptive field size of neurons providing feedforward inhibition to the LGMD has only recently been incorporated into modelling work. Considering this, a model was developed combining features from past literature examples of LGMD modelling with recent anatomical reconstructions based on neural recordings, and a corresponding model of inhibitory networks based on these data. This model, consisting of a simulated LGMD neuron and its relevant input network across the retina, lamina, and medulla, will be tested for its ability to replicate features of LGMD responses to more complex looming stimuli, such as those incorporating trajectory and velocity changes, those presented against moving backgrounds, and those exhibiting limited edge coherence.

I8 Regional specialization to see polarization: a dorsal rim in mantis shrimp?

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Stomatopod crustaceans, often referred to as mantis shrimp, are hailed as the champions of visual complexity. Their compound eyes contain three distinct regions: dorsal and ventral hemispheres and a midband that separates them. These impressive eyes incorporate up to 16 unique photoreceptor types, providing polychromatic color vision as well as linear and circular polarization vision. Previous research on the genetics underlying this complexity revealed that the photoreceptors within the upper margin of the dorsal hemisphere express a unique set of opsins. The expression pattern is redolent of the “dorsal rim” area commonly seen in insect compound eyes, which is used to detect short-wavelength skylight polarization patterns for navigation. Individuals within the stomatopod species *Neogonodactylus oerstedii* are known to use overhead polarization patterns in shallow water for orientation. Thus, they may have convergently evolved this regional opsin expression pattern as a specialization for analysis of celestial polarization - a crustacean "dorsal rim". Previous microspectrophotometric (MSP) analyses poorly characterized rhabdomeric absorption in the dorsal and ventral hemispheres of this species and were insufficient to account for any potential regional specialization. Now, we have extended these earlier studies using MSP, scanning electron microscopy, and histological analysis to search for specialization in hemispheric photoreceptors in *N. oerstedii*. We find that the dorsal-most main rhabdoms absorb light at wavelengths some 80 nm shorter than typical hemispheric photoreceptors, supporting the idea of regional specialization for viewing polarization seen through Snel's window. We interpret our findings to reveal a crustacean analogue of the insect dorsal rim.

I9 Synchrotron source micro-x-ray computed tomography for examining *Lepidoptera* eyes

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Comparative neuroanatomy is critical for determining how different species adapt to their environments through evolution. For example, it is theorized that the organization of ommatidia in compound eyes is optimized for the light levels the animal encounters. However, there is a lack of scalable imaging tools for rapidly mapping microscale eye anatomy. Specifically, many species are not accessible by conventional imaging technologies due to a lack of antibody or genetic labeling tools that permit unbiased imaging of all brain structures. We describe a method using synchrotron source micro-x-ray computed tomography (syn-µXCT) combined with machine learning algorithms for high-throughput imaging of Lepidoptera (i.e. butterfly and moth) eyes. In order to determine whether the brain is getting optimal input from the animal's environment, we measured morphological parameters associated with acuity and sensitivity. We find significant variation between six diurnal Lepidopteran species and the classic measure of optimization, the eye parameter. We conclude that our novel pipeline provides for fast, scalable visualization and analysis of compound eye anatomies that can be applied to any species, enabling new questions surrounding arthropod behavior and ecological niche.

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I10 Visual physiology and behavior of larval stomatopod crustaceans

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Stomatopod crustaceans have one of the most complex animal visual systems currently known. While the adults have historically received the majority of attention, limited work has been focused on larval stomatopods, which have a visual system that is morphologically and physiologically distinct from the adults. Pelagic crustacean larvae generally have compound eyes with a single spectral class of photoreceptor, typically sensitive to the blue portion of the spectrum. However, in this study we tested the hypothesis, based upon recent molecular evidence, that larval stomatopod eyes have multiple spectral channels that are both behaviorally and physiologically relevant. We focused on three species of stomatopods found on Oahu, Hawai'i: *Gonodactylaceus falcatus, Gonodactylellus* n. sp., and *Pullosquilla* n. sp. Eggs from each species were collected and hatched out in the lab. Spectral sensitivity was then measured using ERG recordings under a series of chromatic adaptations. We found that all three species tested had three distinct spectral sensitivity peaks, including a UV peak (340-376 nm), a short wavelength blue peak (455-468 nm), and a long wavelength yellow/orange peak (578-597 nm). Additionally, we completed behavioral tests to explore if there were innate color preferences within the measured physiological ranges. We found that all three species had the strongest preference for UV stimuli, indicating that UV light is a positive cue for larvae. These results are important to our understanding of larval stomatopod spectral diversity, and ultimately add to our understanding of the ontogeny and evolution of the remarkable stomatopod visual system.

I11 Stereopsis in a miniature world: modeling the potential for stereopsis in hunting spiders

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The ability to perceive the spatial structure of one's environment is an integral part of vision. As such, most sighted animals possess the ability to extract depth information via their eyes. Yet, our understanding of depth perception is mostly limited to vertebrate, and largely primate systems. Hunting spiders present an opportune system to expand our understanding of depth perception to invertebrates. Equipped with 4 pairs of camera eyes and unmatched spatial resolution for animals their size, hunting spiders can navigate 3D environments, plan detours, and catch prey from a distance with precise lunges. These behaviors require acute spatial information, but we know very little about how these spiders perceive depth. In particular, the use of stereopsis by hunting spiders has been largely dismissed; their closely-positioned eyes (<< 1cm separation) are assumed to limit the utility of binocular disparity. However, binocular disparity is dictated by viewing distance, as well as eye separation, and the common dismissal fails to account for the short distances at which these miniature animals interact with their environment. We modeled the binocular disparity between eye-pairs of different hunting spider families viewing targets at behaviorally relevant distances. Our results indicated that some spider species have visual systems that allow them to utilize stereopsis and obtain precise depth information, whereas others do not have high enough acuity to detect small disparities. In fact, the binocular disparities for the stereoscopic species were comparable to those of toads, another non-primate system known to utilize stereopsis. This is a first step in our ongoing efforts to explain depth perception across hunting spiders through morphological and behavioral studies.

I12 Performance of apposition compound eyes in the deep sea – a computational model

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The deep sea is unlike any other habitat on earth. Its unusual selective pressures have led to extraordinary variation of animals' visual adaptations. Understanding those adaptations and their effects on vision has been difficult, in part because measuring the capabilities of eyes in deep-sea animals through behavioural and physiological experiments is extremely challenging. However, examining the anatomy of the deep-sea eyes and modelling their visual abilities is comparatively tractable because the visual background is simple and predictable. We developed a new computational model of visual performance in the midwater for apposition compound eyes. The model is based on the output of a detailed microCT analysis and estimates the animals' ability to detect semi-transparent dark and bright objects, as well as bioluminescent point sources. We also incorporated the spatial summation as a strategy to improve visual performance. We used this model to predict the visual capabilities of the double eyes of the hyperiid amphipod *Phronima*. The results show that although both the medial and lateral eyes can detect all types of targets across a broad depth range, the medial eyes outperform the lateral eyes. This suggests that *Phronima*'s medial eyes are useful for a range of visual tasks, not only for looking upwards at dark shapes as was previously hypothesized. We suggest that the unusually strong asymmetry between the medial and lateral eyes of *Phronima* reflects the need for effective vision across a large depth range and their habit of living inside a barrel. The barrel restricts the visual field to its small opening, limiting the benefits of having a large visual field. Our model provides a framework for assessments of visual performance in deep-sea apposition compound eyes.

I13 Multiple mechanisms mediate the suppression of motion vision during escape maneuvers in flying *Drosophila*

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Animals must be able to discriminate self-generated (reafferent) from external (exafferent) sensory input. Otherwise, the former could interfere with perception and behavioral actions. Efference copy mechanisms have been shown to suppress reafferent sensory input in several instances, yet many open questions remain. In *Drosophila*, the optomotor response stabilizes a straight flight path by correcting for unintended deviations, which they sense as visual motion of their surrounding or optic flow. HS cells of the fly are tuned to rotational optic flow and are thought to mediate optomotor responses to horizontal motion. It has been shown that during spontaneous turns, an efference copy influences the membrane potential of HS cells. Here we investigate the influence of an efference copy during looming-elicited evasive turns and how it modulates the processing of horizontal optic flow. We show that looming stimuli themselves can influence the processing of the optomotor stimulus in HS cells. In addition, an inhibitory efference copy is sufficient to suppress responses to preferred direction motion during saccades. We provide evidence that out of the three HS cell subtypes this efference copy acts predominantly on HSN cells, which have dorsal receptive fields. Our results suggest that different HS cell subtypes receive different inputs during saccades and might have different functional roles during flight. In conclusion, our findings support the notion that efference copy mechanisms are highly specialized to fit the sensory perception they target, causing sensory processing to be finely tuned to behavioral context.

I14 Are Lithops "stone" plants? Quantifying chroma and luminance match to rock and soil backgrounds

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Lithops is a genus of succulents that is commonly assumed to be a rare and striking example of the evolution of camouflage in plants. The genus is found across a region of Southern Africa exhibiting a diverse geological history that has left a mosaic of rock types across the landscape, with *Lithops* leaf colour thought to track that of their local rocks, giving the *Lithops* the colloquial name of 'stone plants'. However, whether *Lithops* are camouflaged, and whether it is the stone component of their background they match rather than the soil, has never been tested. We used multispectral images of ~50 *Lithops* populations passed through photoreceptor-noise based

predator visual models to answer these questions. *Lithops* leaf chroma and luminance is correlated with that of their local rocks and soils in visual space, suggesting they are under selection for camouflage. Further, the chromatic distance to local backgrounds (rocks and soils) was frequently lower than predator discrimination thresholds. Luminance distances to backgrounds were often above threshold, although greater variability in background luminance may mean that luminance mismatch does not compromise camouflage. In chroma, we found more support for the stone-plant hypothesis, as *Lithops* tended to be a closer chromatic match to their local rocks than soils. However, in luminance, which substrate *Lithops* was a better match to depended on the subset of rock and soil *Lithops* was compared to. When compared to the average of rock and soil in the background, *Lithops* was closer to soil, but when compared to a nearest subset of these substrates, *Lithops* was closer to rock. This work is one of the first quantitative examples of camouflage in plants, and shows that *Lithops* are deserving of their 'stone plant' epithet.

I15 Colour vision in the dark: retinal computations underlying chromatic discrimination in low illumination

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Colour vision in very dim light is out of reach for most vertebrates because the rod photoreceptors that mediate scotopic vision usually come in a single "green" spectral flavour, categorically precluding spectral comparisons. Amphibians are the only known exception, with frogs and some salamanders possessing an additional "blue" rod, and recent behavioural experiments showed that frogs and toads can indeed make spectral comparisons near the absolute visual sensitivity threshold in specific behavioural contexts. How is this "colour vision in the dark" achieved at a circuit level? To address this broad question, we are using high-density multielectrode array recordings of retinal ganglion cells (RGCs) from adult *Xenopus* retina. We probe responses with a battery of "greyscale" and "coloured" stimuli across different brightness regimes to identify RGCs that are informative about wavelength in photopic, mesopic and scotopic conditions. Preliminary data indicates that most RGCs are On-Off, in line with previous work mostly on salamanders. Moreover, we note the presence of diverse achromatic and colour opponent RGCs in photopic and mesopic conditions. As light levels decrease, many RGCs become achromatic, consistent with dominant drive from a single rod-type, whereas a few of them appear to remain informative about wavelength even in the darkest-tested conditions. These typically On-Off cells are driven by inputs spectrally consistent with green-rods, but on top of that their On responses are weakly but consistently suppressed at shorter wavelength. From here, we speculate that blue-rods might feed into the "regular" green-rod circuitry via an overall On-suppressive circuit, offering a potential explanation for colour biased behavioural responses under low illumination.

I16 Vision in sturgeons: evolution of the opsin genes and how to see without rod cells in retina

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Opsin genes encode for opsin proteins, which are responsible for light reception in the rod and cone cells in the retina. Fishes in general show numerous visual adaptations. Most of the fish lineages have also experienced additional whole genome duplications (WGDs) on top of the two vertebrate rounds. WGDs represent substantial evolutionary force, since they provide "genetic raw" material and in general increase genes number. There were at least three WGD events during sturgeon evolutionary history independent of the teleost-specific genome duplications. In this study we identified opsin genes of several sturgeon species and investigated their expression level in sturgeon subclades with different WGD history and living in different habitats. We found an exceptionally modified species, the Starry sturgeon (*Acipenser stellatus*), which does not express any rod opsins, and hence seems to lack rods in the retina. To visualize cone cells and their putative mosaic pattern, we applied

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FISH (fluorescence in situ hybridization) on the *A. stellatus* retina. Further, in Sterlet (*A. ruthenus;* evolutionary diploid) and the Siberian sturgeon (*A. baerii*; evolutionary tetraploid), we had the opportunity to compare opsin gene repertoire and their expression level between pure species and their hybrids, normoploids and specimens with manipulated ploidy level, and, lastly, between pigmented and albino specimens.

I17 Spectral and polarisation information processing in the stomatopod visual system

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Stomatopods (mantis shrimps) have a unique visual system possessing perhaps the most diverse set of photoreceptors among all animals. Each of their compound eyes is divided into 3 regions by an equatorial band of 6 rows of enlarged ommatidia, called the midband. In a neatly segregated manner, these different eye-zones mediate luminance, colour (12 channels 300-710nm), linear (4-directions) and circular polarization (L and R) vision. Although their visual system has been well investigated anatomically and behaviorally, there is little understanding of how they process their 20 channels of visual information. To fill this gap, we used intracellular recordings to examine the visual processing mechanisms in the first optic lobe, *i.e.,* the lamina, of stomatopods. We also used behavioural experiments to examine colour discrimination and colour constancy under both white light and coloured illumination. Our electrophysiological results found lamina interneurons showing either colour or CP opponent properties, suggesting that spectral and CP opponent processing exists early on in the stomatopod visual system. Behavioural results showed that their colour discrimination performances did not differ under different illuminations, which indicated that each midband rows 1-4 is likely to interpret colour information individually with an opposing system capable of colour constancy. These results are up for discussion relative to the ecology and lifestyle of stomatopods and support both old and newer hypotheses on their information processing system.

I18 CRISPR/Cas9-mediated knockout of *Amlop1* opsin reduces color learning efficiency of honeybees in a passive-avoidance task

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Honey bees are endowed with the capacity of color vision thanks to three types of photoreceptors that are maximally sensitive in the ultraviolet, blue, and green domains owing to the presence of corresponding opsin types. While the behavioral aspects of color vision have been intensively explored, the molecular underpinnings of this capacity are largely unknown. To investigate the functional differences between two types of green opsins (*Amlop1* and *Amlop2*), we developed a CRISPR/Cas9 approach and successfully created *Amlop1* and *Amlop2* mutant bees, as well as *white*-gene mutants as a control for the efficiency of our method. We tested our mutants using a conditioning protocol, in which bees learn to inhibit phototaxis towards chromatic light based on electric-shock punishment. *White* and *Amlop2* mutants could successfully associate a color with the electric shock punishment, while *Amlop1* mutants failed to do so. Accordingly, *white* and *Amlop2* mutants exhibited an aversive memory for the punished color, whereas *Amlop1* mutants exhibited no memory. These results indicate that responses to blue light, which is also partially sensed by green receptors, are mediated mainly by compound-eye photoreceptors containing *Amlop1*, but not by the ocellar system where *Amlop2* is expressed. We discuss these findings in terms of chromatic vision and in terms of the consequences that the induced mutation might have on other mechanisms of neural signaling.

I19 Damsels in colour: adaptations of the visual system and colouration during the development of coral reef damselfishes (*Pomacentridae*)

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With more than 405 described species, damselfishes (Pomacentridae) are one of the most abundant and species-rich coral reef fish families. Damselfishes differ greatly in ecology, behaviour and in colouration, which has recently been shown to correlate with visual gene (opsin) expression in adult fishes. However, little is known about vision in earlier stages including how opsin gene expression might change during major ecological transitions such as when larvae settle on the reef or when fish mature from juveniles to adults. Moreover, many damselfishes show extreme colour changes during these transitions, but the ecological significance of these colour changes remain mostly elusive. Using a multidisciplinary approach, including bulk retinal RNA sequencing (RNAseq), visible and ultraviolet calibrated (UV) photography, spectrophotometry, and phylogenetic comparative analyses, we investigated the development of the visual system and colouration in damselfishes from the Great Barrier Reef, Australia. Preliminary data from the RNAseq approach has revealed both interspecific and ontogenetic differences in opsin gene expression, with some species already showing adult expression profiles, while others rely on a different opsin gene repertoire during earlier stages. Few species express multiple copies of the UV-sensitive *SWS1* opsin, which likely support intraspecific communication in UV. Supporting the communication hypothesis, UV-photography revealed species expressing multiple *SWS1* genes develop 'hidden' UV body patterns as they grow. Ongoing experiments will continue to investigate the possible link between opsin gene expression with ontogenetic colour changes both in the visible and the UV and what role phylogeny plays in the visual development of these fishes.

I20 The retinal basis of vision at the origin of vertebrate life

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Despite the mass species diversification during the Cambrian explosion (>500 million years ago) and the occurrent wide range of eye shapes and sizes thereafter, the basis of retinal vision of all vertebrates is strikingly based on the same basic circuit motif, comprising of five neuronal classes arranged into three nuclear and two synaptic layers. And yet, owing to the ever-narrowing focus on model species we currently have a detailed understanding of the retinal circuits' structure and function in only a handful of species, including in rodents, primates and to a lesser extent also in zebrafish. If mice possess more than 40 functionally distinct output channels from the retina to the brain, how many and which are mice- or rodent-specific, reflect the specific organism's visual space statistics or behavioral repertoire, or are evolutionary conserved or constrained? To address these types of broad questions, we seek to 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. Here we present our ongoing efforts to establish the first high-throughput recordings of retinal ganglion cells of an elasmobranch retina, using the catshark *Scyliorhinus canicula* as a model. Using multi-electrode arrays we record from hundreds of cells in parallel during the presentation of a broad arsenal of visual stimuli that systematically probe key principles of spatial, temporal, and spectral processing. We find that the picture of "poor vision" in sharks is far from the truth.

I21 Opsin repertoire and light-mediated behaviors of the starlet sea anemone, *Nematostella vectensis*

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Cnidaria (corals, anemones, jellies) are the sister group to all bilaterally symmetric animals. The common ancestor of Cnidaria and Bilateria possessed many of the gene families associated with modern bilaterian complexity, such as visual system components like opsins. Opsins have diversified and and opsin-expressing cells have been incorporated into a range of visual systems from simple to complex in both lineages. Less-complex visual systems are important to our understanding of eye evolution and function, but have often been overlooked, especially in Cnidaria. The starlet sea anemone, *Nematostella vectensis* is an excellent lab animal, however little is known about its sensory biology. Despite lacking an eye and a central nervous system, *N. vectensis* exhibits a variety of light-mediated behaviors and has a large expansion of opsin genes in its genome. Before establishing which opsins are responsible for photosensory behaviors in *N. vectensis,* a baseline characterizing the opsins and behaviors is an important first step. I present the first complete opsin repertoire of this sea anemone, which has 30 opsins, the most of any anthozoan so far investigated. These fall into three distinct opsin clades, and are expressed dynamically throughout development and in adults. Light-mediated behaviors include circadian patterns in locomotory behavior, scrunching, larval swimming, and spawning. The only characterized behavior is locomotory circadian rhythms while the others are poorly studied. Here I characterize larval swimming and scrunching under a variety of intensities and spectral conditions. Current and future work involves knocking out individual opsins and assessing their function in light behaviors, as well as generating opsin reporter lines.

I22 Chromatic motion sensitive neuron in the yellow Japanese swallowtail butterfly

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The optokinetic response elicited by wide-field motion is an essential response for flight control, which is based on the achromatic system in many insects. But in *Papilio* butterflies, stripe patterns with two iso-luminant colors, i.e. of equal brightness to the butterfly and thus only containing chromatic contrast, elicit strong optokinetic responses. To reveal the neural basis of chromatic motion vision, we performed single-cell recordings followed by dye injection. We encountered many neurons sensitive to black-and-white stripe patterns moving vertically in the second visual ganglion, the medulla (Me). These neurons have branches with varicosities in the Me and smooth branches spreading in the contralateral ventral lateral protocerebrum (VLP); their morphology suggests a feedback function to the Me from contralateral VLP. In fact, the neurons' motion-sensitive responses disappeared when we occluded the contralateral eye while recording, confirming their feedback function. Moreover, the neurons responded to motion when we used stripe patterns of two iso-luminant colors. A series of experiments where we systematically controlled the chromatic and lightness contrasts revealed that the neurons' response profile was similar to that of the behavioral response. Presumably, by using chromatic information in the motion vision pathway rather than depending solely on achromatic information, the butterfly can increase its sensitivity to motion.

LEARNING, MEMORY AND COGNITION I

J1 State-dependent judgement biases in bees demonstrated using an active choice task

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Is the glass half empty or half full? This simple "test" captures the role that inner state might have on cognitive perception. Those in a more positive state will define a glass as half full, while those in a negative state will have more pessimistic judgments. Such a judgement bias can serve as a good proxy in measuring affective or emotion-like states in animals. While a few studies have shown judgement biases in bees, the experimental design has allowed for alternative interpretations of the results. We, therefore, tested for judgement biases in bees in a more reliable way using an active choice task. We trained bees to use colour cues to fly to distinct locations, where each location was associated with either a high- or a low-quality sugar reward. After learning this discrimination, bees were randomly allocated to one of three groups. Two groups were subjected to a negative affective state induced by either shaking them on a Vortex or trapping them by a robotic arm. The third group served as a control. We then presented bees with a series of ambiguous colours of intermediate value between the two conditioned colours and recorded the first choice made by each bee. Choices of reward chambers in the locations previously conditioned with high- or low-quality rewards were noted as optimistic and pessimistic choices, respectively. As predicted, on the trials with ambiguous cues, bees subjected to a negative state were less likely to choose reward chambers associated with high reward, and were thus less optimistic compared to the control group. Notably, manipulations did not affect the overall motivation to feed and the learned colour discrimination. Altogether, these findings add strong evidence showing that the affective state influences cognitive processing in insects

J2 Mushroom body output population activity allows for odor-cued behavioral prediction

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Animal behavioral decisions are based on the value of external stimuli and especially feeding behavior is strongly influenced by odor valence. Particularly in nocturnal insects olfaction is of importance to find food sources and categorize them appropriately. The mushroom body output acts as integrator of several kind of information coming from different areas and plays a relevant role in valence coding. To understand how neuronal representation of odor valence at the mushroom body output level determines feeding behavior we performed extracellular recordings of the mushroom body output region in fixed cockroaches (*P. americana*). Simultaneously we recorded the movement of the mouthparts during odor stimulation in individual animal. The cockroaches showed a feeding response (maxilla-labia response) almost exclusively to food related odors and similar odors increased neuronal activity. With this data we were able to fit a logistic regression classifier that predicted maxilla-labia response with about 90% accuracy. This led to our hypothesis that the mushroom body output is involved in the decision making process based on odor valence.

J3 Multimodal learning modulation by biogenic amines in bumblebees (*Bombus impatiens*)

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The neuronal process of multimodal integration allows signals from two or more sensory modalities to be processed together. Multimodal integration influences all behaviour, being especially relevant in tasks requiring learning and memory skills, such as foraging or pollination. The neuronal substrate for learning and memory is modulated by neurotransmitters such as biogenic amines. These, regulate synaptic interactions and, depending on concentration and brain location, affects behaviour or sensory systems' sensitivity. In bees and bumblebees dopamine (DA) has an inhibitory effect on olfactory memory, while octopamine (OA) modulates the reward: blocking OA prevents olfactory learning while increasing its availability might improve it. While less studied, visual learning neuromodulation might be influenced by OA and DA signalling through appetitive sucrose signalling or by the colour + sucrose reward in the brain. However, the role of biogenic amines during multimodal learning is unknown. In this study, we analysed the learning and memory performances of bumblebees treated with biogenic amines (OA and the DA receptor agonist 6,7-ADTN) during olfactory, visual, and multimodal (O+V) stimulation. We found an overall learning enhancement effect of OA is concentration-dependent across all modalities. Low concentrations of the DA agonist, induced the highest inhibition during olfactory while visual acquisition was inhibited across concentrations. Interestingly, multimodal learning escaped such inhibition. Our data suggest that biogenic amines modulate uni and multimodal learning differentially. Both learning enhancement and inhibition depend not only on the identity and concentration of the biogenic amines but on the complexity of the learned stimuli.

J4 Musicality influences active sensing behavior in a freely-moving frequency discrimination task

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Our perception is based on active sensing, i.e. the relationship between self-motion and resulting changes to sensory inputs. Yet, traditional experimental paradigms are characterized by delayed reactions to predetermined stimuli. To investigate richer behavioral responses, we developed the Sensory Island Task for humans (SITh), a freely-moving search paradigm to study auditory perception. In SITh, subjects navigate an arena in search of an auditory target, relying solely on changes in the presented stimulus frequency, controlled by closed-loop position tracking. A "target frequency" was played when subjects entered a circular sub-area of the arena, the "island", while different frequencies were presented outside the island. Island locations were randomized across trials, making stimulus frequency the only informative cue for task completion. Two versions of SITh were studied: binary discrimination, and gradual change of the stimulus frequency. The latter version allowed determining frequency discrimination thresholds based on the subjects' report of the perceived island location. Subjects exhibited similar thresholds as reported in traditional 'stationary' forced choice experiments after performing only 30 trials, highlighting the intuitive nature of SITh. Notably, subjects spontaneously employed different search patterns, and frequency discrimination performance depended on the pattern used. The best performing strategies, characterized by adapting search patterns incorporating the acoustic feedback, were preferred by the most musically experienced subjects. Overall, we demonstrate that the use of an ecologically driven paradigm can reproduce established findings while simultaneously providing rich behavioral data for the description of sensory ethology.

J5 Female brain molecules orchestrate mate memory to avoid cheater males

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Female mate choice is a powerful selective pressure that shapes the evolution of extravagant male sexual traits and male mating strategies. Combining proteomic analyzes with behavioral tests, we studied female *Tenebrio molitor* brain during mate choice and consider the implications for sexual selection strategies. *T. molitor* is a polygamous species, attractive males with a high pheromone production include honest males in good condition and deceptive males in poor condition. We found the presence of methyltransferases (MTase) related to memory in females mated with attractive males. In mammals, mating partner memory involves epigenetic proteins, oxytocin and vasopressin, but in insects, inotocin could be involved. We asked whether females mated with preferred males a) remembered their partner and b) whether inotocin is involved. a) We found that most females preferred to mate with their previous partner over a new male. This memory persisted for 5 days, but if females constantly evaluate males, persists longer. We applied a MTases inhibitor to females before the memory trial and we found that treated females lost the mate preference. b) Females treated with inotocin were less likely to mate, but those who mated were more likely to remember their partner. We hypothesized that female mating memory is a mechanism to avoid cheating males. We tested the females when their previous partners had lower condition than during the first encounter and lower than the new male. Females did not exhibit mate preference. We propose this memory may have evolved to enable females to avoid previous partners in a subsequent encounter if they falsely advertise their condition. Exploring these mechanisms in invertebrates will improve our understanding of mate choice cognition evolution.

J6 Neuro-morphology and molecular changes of sex pheromone learning in butterflies

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Insects can modify their sexual preferences and behaviors based on previous social interactions or the experience of visual, olfactory, gustatory, or auditory signals. These early experiences can potentially affect the evolution of sexual traits, patterns of assortative mating and ultimately speciation. The underlying mechanisms associated with this behavioral plasticity, however, are still unclear. We previously showed that

female *Bicyclus anynana* butterflies can learn to prefer new male sex pheromone after a short exposure to this signal. Here, we provide a set of candidate genes and identify brain structures that are affected by the female new experience. We compared the transcriptome and the methylome of naïve and exposed female antennae and brains, as well as the volumes of the neuropiles in their antennal lobes. We identified olfactory receptors being differentially expressed or methylated in the antennae, and the volume of glomeruli changing in female brains after experiencing the new pheromone blend. The detailed molecular and neurological pathways by which these candidate genes and structures affect the behavior of females remains to be explored.

J7 Morphological differences and task specialisation: do polymorphic ant workers differ in nestmate recognition abilities?

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Many ant species consist of polymorphic workers that differ in size and shape and these differences are often associated with specialised roles within the colony. Nestmate recognition is an essential task, which maintains the integrity of the colony by preventing unrelated individuals from exploiting the nest. The ability to detect nestmates and non-nestmates relies on blends of cuticular hydrocarbons (CHCs), with each colony possessing their own signature blend. The detection of these CHCs relies on specialised antennal sensilla (basiconica) that project onto a specific area in the antennal lobe. Using the highly polymorphic harvester ant, *Messor barbarus*, we investigated whether distinct worker morphs differ in these specialized structures and whether this variation correlates to the ability to perform nestmate recognition. Our study aims to elucidate how workers may differ at the behavioural and morphological level which gives rise to task specialisation within the nest.

J8 Investigating visual coding and memory in the honey bee brain

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Honey bees associate olfactory and visual stimuli with a sugar reward both in a natural context and in controlled protocols in the laboratory. They provide, therefore, an ideal model for the neuroethological study of sensory coding and memory formation. While coding principles of olfactory perception and learning have been extensively studied in bees, allowing us to acquire some insight on the nature of an olfactory memory trace, our knowledge of the neural correlates of visual coding and learning remains very limited. By localized injection of a calcium sensitive probe onto the neural tracts projecting from the optic lobe to the protocerebrum, we were able to reliably label the lobula neurons. Here, we report the first results obtained through coupling protocols of visual stimulation with *in vivo* calcium imaging analysis of the honeybee lobula. Thus, we provide a model for the investigation of how visual stimuli are represented in the honeybee brain, and how such representation evolves with learning.

J9 Neural signature of visual learning under virtual-reality conditions in the honey bee

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Honey bees display a rich behavioral repertoire, in which appetitive learning and memory play a fundamental role in the context of foraging activities. To study these capacities and their underlying neural mechanisms, we developed a 3D virtual reality (VR) that allows a precise control of visual experience during visual learning. Using this environment in which a tethered bee walks stationary and makes decisions based on color information in closed-loop conditions, we performed *ex-vivo* analysis of immediate early gene (IEG) expression

in specific brain areas, comparing learners and non-learners. Using both 3D VR and a more restrictive 2D version of the same task we tackled two questions, first what are the brain regions involved in visual learning? And second, is the pattern of brain activation dependent on the learning task? Our results show that learner bees that solved the task in 3D exhibited an IEG upregulation in the calyces of the mushroom bodies. Yet, a different pattern of IEGs expression was found in learner bees conditioned under the 2D environment. In this case, an IEG downregulation was found in the optic lobes and in the calyces of the mushroom bodies, suggesting an inhibitory neural trace. Coupling VR and *ex-vivo* IEG analysis thus allowed us to show that visual learning creates a distributed memory trace along the visual pathway that is dependent on the nature of the learning task.

J10 Charactering the roles of neuropeptides in non-associative learning

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Non-associative learning is the simplest form of learning that is thought as the "cognitive building blocks" for higher learning to occur. To better understand non-associative plasticity and its cellular and molecular mechanisms, our work focuses on neuromodulatory signalling pathways underlying these forms of plasticity. First, we found that *egl-3* and *egl-21* mutants deficient in a number of neuropeptides showed learning deficits in three forms of non-associative learning, sensitization, habituation, and dishabituation, suggesting neuropeptides play a key role in non-associative learning. Further, we found that FLP-20/FRPR-3 signalling mediates sensitization but not dishabituation of the same response, and that the same neuropeptides differentially affect sensitization of response duration and response speed. These data suggest different forms of non-associative plasticity are mediated by different cellular and molecular components. We are in the process of testing a range of neuropeptide gene mutations to examine how they influence non-associative learning. This project will generate data to better understand how neuropeptide signalling mediate different forms of non-associative plasticity.

J11 Deconstructing collective cognition in *Drosophila*: neurobehavioural mechanisms of social and asocial learning

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The cognitive system has an important function in filtering the information an animal receives from the environment and in determining the appropriate responses, therefore affecting animal performance at the behavioural level. Currently, there is a debate on whether there is a specialization of cognitive mechanisms for the processing of asocial and social information. Cognition in *Drosophila* is usually studied using olfactory collective conditioning. However, this approach does not allow to disentangle between social and asocial learning. Nonetheless, many studies using this paradigm have already identified genes that disrupt the different phases of memory storage, as well as the input and encoding of information. In order to disentangle to disentangle the social and asocial components in collective learning and to what extent the same genes are implicated in both learning types, we developed new paradigms of social and asocial olfactory conditioning using the classic T-Maze *Drosophila* learning set-up. We will present the preliminary results of these tests in established mutant lines for memory in *Drosophila* (e.g. radish mutant line which is associated with anaesthesia-resistant memory).

J12 Information integration for nutritional decision-making in desert locusts

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Swarms of the migratory desert locust can extend over several hundred square kilometres, and starvation compels this ancient pest to devour everything in its path. Theory suggests that gregarious behaviour benefits foraging efficiency across relatively wide ranges of spatial food distributions. However, despite the importance of identifying the processes by which swarms locate and select feeding sites to predict their progression, the role of social cohesion during foraging remains elusive. We investigate the evidence accumulation and information integration processes that underlie locusts' nutritional decision-making by employing a Bayesian formalism on high-resolution tracking data of foraging locusts. We tested individual animals and groups of different sizes in a 2-choice behavioural assay in which food patch qualities were either different or similar. We then predicted individual locusts' decisions based on personally acquired and socially derived evidence by disentangling the relative contributions of each information class. Our study suggests that locusts balance incongruent evidence but reinforce congruent ones, forming stronger opinions when evidence aligns. We provide new insights into the interplay between personal experience and social context in locust foraging decisions which constitute a powerful empirical system to study local individual decisions and their consequent collective dynamics.

J13 Judgment bias influences the neurobiological control of behaviour

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Recent literature on judgment bias suggests that the tendency to behave or react towards ambiguous stimuli is relatively stable and individual-specific, which implies that judgment bias may reflect a personality trait. However, the neurobiological mechanisms and behavioural consequences associated to inter-individual variation in judgment bias remain largely unresolved. Here we explore the potential role of dopaminergic and serotonergic signalling in the regulation of the behavioural responses displayed in a novel environment of both pessimistic and optimistic zebrafish (*Danio rerio*). Fish were first screened in a judgment bias paradigm and classified in optimistic/pessimistic categories. Following this, optimists and pessimists were subjected to an unpredictable chronic stress protocol. Finally, all fish were assessed in the novel tank test (NTT) before brains were sampled for quantification of monoamine neurochemistry. Monoamine analyses revealed an interaction between the dopaminergic system and judgment bias in the regulation of risk taking and exploratory behaviours associated to the NTT, which were specifically modulated in the hypothalamus and brain stem. Our results also revealed that serotonergic signalling modulating anxiety-related behaviours associated the NTT is affected by chronic stress at the optic tectum level.

J14 Protection of bumble bees using phytochemicals against impairments induced by the neuropesticide fipronil

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Food security is a growing global issue tied to the ongoing decline of populations of pollinators, such as bees. The exposure to neuroactive pesticides, such as fipronil, causes cognitive impairment, presumably due to degeneration of main brain regions, including the mushroom bodies and the antennal lobes. We have found that prophylactic administration of the flavonoid rutin induces protection in olfactory learning in the bumble bee B. impatiens against administration of fipronil and imidacloprid. Hence, here we aimed to evaluate whether the observed improvement is underlied by a structural protection. We fed individual foragers of B.

impatiens one of two phytochemicals, namely rutin and p-coumaric acid. Then, we administered realistic doses of fipronil and conducted behavioral essays (olfactory learning and memory) and neuroanatomical analyses (overall brain structures, microglomeruli within the lip). We predict that the administration of fipronil will induce neurodegeneration within the mushroom body calyces and that the administration of both phytochemicals will lead to behavioral protection as well as to a reduction in neurodegeneration. Our ongoing analyses follow the previous observed patterns and will enable us to test these predictions and will provide further insights into the use of phytochemicals as inductors of protection against impairments induced by pesticides. Overall, our results will contribute as an alternative to the ongoing dilemma between the use of insecticides and the decline of pollinators.

J15 Looking for immediate early genes as neuronal activation markers in the cephalopod mollusc *Sepia officinalis*

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Immediate early genes (IEGs) are genes which are expressed rapidly and transiently in brain neurons in response to various stimuli. Imaging IEGs is a commonly used tool to study neuronal activation, especially in learning and memory tasks. Recent behavioural studies indicate how proficient common cuttlefish are in these aforementioned tasks, however the neural substrates associated to these abilities have yet to be fully explored. We aim to validate suitable IEGs and appropriate in situ hybridization (ISH) protocols to perform functional studies of the cuttlefish brain. Three genes (cFos, Uch and Egr1) were selected as potential IEG candidates, their orthologs were identified in *Sepia officinalis* transcriptomes and cloned for RNA probe synthesis. Their expressions were compared both in nervous tissues (by ISH) and quantitatively (by qPCR) between two groups of one-week-old cuttlefish. The stimulated group was exposed to stimulations involving multiple sensory modalities (light flashes, odours, tactile stimulation and handling), whereas the control group was kept in their usual captive conditions under dimmed light and without any stimulations. Our first results indicate that these three IEGs are expressed in the cuttlefish brain and activated by sensory stimulation, showing at minima small differences. Egr1 noticeably produces the greatest differences in neuronal activation between test and control groups. Its expression is found in diverse brain areas, making it the best candidate for our upcoming studies on the neural substrates of cuttlefish memory.

METABOLISM, BIOLOGICAL RHYTHMS AND HOMEOSTASIS I

K1 Dietary effects on the activity of insulin producing cells in *Drosophila*

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Insulin signaling plays a key role in controlling metabolic homeostasis. In addition, insulin is heavily implicated in processes underlying reproduction, aging and stress resistance. Insulin producing cells (IPCs) in *Drosophila* are functionally analogous to mammalian pancreatic beta cells and produce different *Drosophila* insulin like peptides (DILPs) during different stages of a fly’s lifespan. In the adult fly, release of these DILPs is dependent on nutrient availability. Nutrient sensing is vital for metabolic control. Fuel substrates such as glucose serve immediate energy demanding processes such as locomotion, mating, etc. *Drosophila* IPCs are hypothesized to play a role in sensing hemolymph glucose levels cell-autonomously. Therefore, we set out to perform an *in-vivo* electrophysiological study of nutrient sensing in Drosophila IPCs. To label IPCs for patch-clamp recordings, we used a Dilp2-Gal4 driver line to express GFP in IPCs of the adult *Drosophila* brain. Using these flies, we performed targeted *in-vivo* patch-clamp recordings from IPCs while perfusing the brain with artificial hemolymph containing different concentrations of glucose and other sugars. Furthermore, we exposed flies to different diets and quantified their effects on IPC activity. In addition to quantifying the different dietary effects, we explored the effects of aging on IPC activity. We found that nutritional state and aging strongly affected IPC activity.

However, varying extracellular glucose levels caused no significant changes in their activity. This indicates that, although dietary sugars have strong effects on IPC activity, IPCs are not sensitive to local changes in extracellular glucose levels *in-vivo*.

K2 Behavioral state-dependent modulation of insulin-producing cells in *Drosophila*

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Insulin plays a pivotal role in metabolic control, neuromodulation, and aging, but its release dynamics are not well understood. We used *Drosophila melanogaster* to study effects of locomotion on the activity of insulin-producing cells (IPCs). Using a combination of *in-vivo* patch-clamp recordings, calcium imaging, and optogenetics, we found that IPCs were inhibited during walking and flight. This modulation was graded, such that the inhibition was stronger during flight – the more energy-demanding behavior. A resulting decrease in insulin levels would support the mobilization of fuel stores and the suppression of anabolic processes during locomotion. IPC activity was increased immediately after cessation of locomotion. This rebound could contribute to replenishing muscle glycogen stores. Surprisingly, IPC modulation preceded the onset of locomotion, suggesting a feedforward mechanism impinging on IPCs. This was further supported by *ex-vivo* recordings combined with optogenetic activation of motor circuits, which revealed that IPC inhibition neither requires actual behavior, nor decreased blood sugar levels – a simple motor command was sufficient to inhibit IPCs. In a nutshell, we add the behavioral state to the list of factors regulating IPC activity in *Drosophila*. These rapid changes in IPC activity precede locomotion and may serve an increased metabolic demand. Moreover, high insulin levels are known to decrease the sensitivity of olfactory sensory neurons in flies. Hence, the inhibition of IPCs could lead to a disinhibition of olfactory sensory neurons, which could increase the likelihood of locating food sources during locomotion. Thus, behavioral state-dependent IPC modulation might enable differential sensorimotor processing.

K3 Neural circuits and neuromodulators that influence waking arousal in zebrafish

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Organisms alter their behaviors to match dynamic environmental cues. The inability to modulate arousal appropriately is a shared symptom of several neurological disorders, while neural circuits that control arousal in different environmental contexts are largely unknown. To study arousal, we use a behavioral paradigm where transient exposure to water flow results in a lasting, heightened arousal state characterized by increased visual sensitivity and locomotion. To identify the neurons required for arousal, we systematically ablated distinct neural populations by crossing Gal4 enhancer trap lines to UAS:nitroreductase. Ablation of neurons in the Gal4 y294 pattern – labeling the rostral hypothalamus, dorsolateral hindbrain, thalamus, habenula, and dorsal raphe – led to muted flow responses. Ablation of neurons just in the habenula or in the dorsal raphe did not result in any changes in the flow response, suggesting these populations alone are not required for arousal. To determine which neurons within the y294 pattern are important, we have created a genetically encoded construct, UAS:dl5, to perform spatially selective ablations using near infrared light. We have also constructed UAS:CaMPARI2 to allow us to identify active neuronal populations during post-flow arousal. Finally, we identify potential neuromodulators involved in flow-induced arousal. Exposure to drugs that block dopamine receptor 2A led to muted flow responses. However, gene knockdowns of individual dopamine receptor 2 genes and ablations of dopaminergic cells did not alter the arousal response, suggesting the involvement of other neuromodulators. Further investigation will determine which neurons within the y294 pattern are involved in arousal modulation and which neuromodulators regulate arousal.

↑UP

K4 Sex differences in circadian clock neuron network resilience in *Drosophila melanogaster*

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A growing body of evidence indicates the existence of sex differences in the circadian system. Drosophila is a powerful model system in which to study the neuronal basis of sexual dimorphism in timekeeping thanks to its well-characterized circadian clock neuron network and highly conserved physiology. Circadian clocks in Drosophila melanogaster comprise of about 150 neurons distributed bilaterally in the brain and orchestrate the timing of multiple behavioral outputs. A subset of these neurons, the small ventral lateral neurons (s-LNv) secrete the neuropeptide Pigment Dispersing Factor (PDF) which functions as a synchronizing factor in the circadian network, with complex effects on downstream neurons, such as required for maintaining rhythmicity in some clock cells and adjusting the phase of the molecular clock in some other cells. Here, we provide evidence of sex differences in the fundamental properties of the circadian system in male and female flies. We employed mutants as well as clock neuron-specific manipulations of the clock network synchronization and found that female sleep/wake cycles are less affected by manipulations of the PDF signaling pathway. We also examined the effect of the loss of PDF on the molecular oscillations of the core clock proteins, Period (PER) and Timeless (TIM), in different neuronal classes within the clock neuron network of males and female flies. We provide evidence supporting the notion that the female circadian network is less sensitive to genetic perturbations that impact the network synchronization.

K5 Effect of dietary P:C ratio and amino acid ingestion in *Crematogaster scutellaris* ant behaviour

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In the last decades, the Geometrical Framework of Nutrition has shown how the ratio between protein (P) and carbohydrate (C) in the diet might have important effects on animal survival, reproduction, and fitness. High protein diets reduce lifespan in many organisms, including ants. In some ant species, a low carbohydrate diet seems to decrease the aggressive behavior and activity of ant colonies. In this study, we have used workers of the Mediterranean ant *Crematogaster scutellaris* to evaluate if the P:C ratio and the complexity of the dietary protides (free amino acids, AA, or whole proteins, WP) modulate their social and foraging behavior. First, we have followed the survival and physiological state of workers fed on diets with different P:C ratios (1:25, 1:10, 1:5, 1:2, 1:1) and complexity of protides, and we have confirmed that, as in other ant species, higher P:C ratios reduce lifespan and lipid reserves in ant workers. Free amino acids are more toxic than whole proteins in high protein diets, but not in high carbohydrate ones. In this experiment, we also observed that workers tended to forage more high protein diets made of whole proteins than on those made of free amino acids. In order to test whether their ability to follow trails was compromised, we fed some workers on 1:1 WP:C, 1:1 AA:C and 1:25 WP:C and 1:2 AA:C and test them on pheromone trails, finding the behaviour was not affected by the diet. Second, we selected some P:C ratios (1:25, 1:5 and 1:1) to evaluate if their aggressivity to other species and their intracolony recognition was affected by the diet. None of the aggressive behaviours observed were modified by the diet, and only the antennation frequency to nestmates seemed to be different in one of the diets. In conclusion, the physiological state and the foraring behaviour changed depending on the diet, but not other relevant behavioral change was observed, at least in our experimental design.

K6 Cellular and molecular underpinnings of hibernation anorexia

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Hibernation is an effective strategy some animals use to survive cold, inhospitable environments and food scarcity. Regulation of hunger is essential for hibernator survival, as premature emergence from the burrow to seek food may dysregulate other dependent processes and increase the risk of predation. Thirteen-lined ground squirrels hibernate for 5–7 months out of the year. During this time, they do not cache food or eat food that is offered. Instead, energy during hibernation is supplied by body fat amassed during the summer. Therefore, squirrels demonstrate seasonal, self-induced anorexia. The mechanism and function of hibernation anorexia are largely unknown. Here, we investigated mechanisms of anorexia during hibernation by examining the role of peripheral hormones and their interaction with the arcuate nucleus (ARC) of the hypothalamus, a brain region that coordinates homeostatic feeding. We found that hibernating animals are resistant to the hunger-inducing effects of ghrelin, a hormone produced in the stomach that normally induces feeding. ARC "hunger" neurons, which contain ghrelin receptors, fail to be activated by ghrelin during hibernation. This ghrelin resistance is reversible, as ghrelin sensitivity is recovered during the summer season. Furthermore, we demonstrate that squirrels have reduced hypothalamic thyroid hormone triiodothyronine (T3) during hibernation. We predict that central hypothyroidism reduces the excitability of AgRP neurons, such that they are unable to be excited by endogenous ghrelin to induce hunger. In support of this hypothesis, hypothalamic infusion of T3 is sufficient to rescue hibernation anorexia. Therefore, we have uncovered seasonal ghrelin resistance and central hypothyroidism as mechanisms which enforce hibernation anorexia.

POSTER SESSION II
(Thursday, 16:30–19:30)

SPATIAL ORIENTATION AND NAVIGATION II

A24 Representation of visual landmarks in mouse primary visual cortex during navigation in virtual reality

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Navigation is one of the key functions for the visual system, yet how the environment is represented in the visual cortex during navigation is not well understood. Taking advantage of rodent virtual reality (VR) systems, recent work has shown that non-visual signals such as spatial signals are represented in the visual cortex, characterised as "spatial modulation" of neural responses. In previous work, spatial modulation was measured as the difference between the neural response to two identical landmarks in different spatial locations. In the current study, we used landmark manipulations in VR to uncover spatial and non-visual components of the response, which is an approach with less confounds. We recorded population activity in V1 (475 reliable units) using large-scale extracellular probes while mice (N=7) ran in a VR corridor, in which there were 4 visual landmarks. Once mice had been acclimatised to the virtual environment, we omitted, or swapped the location of some landmarks to characterise non-visual signals in the population response. When we segregated the units into units that responded around the landmark (landmark-tuned) and units that responded in-between landmarks (non-landmark tuned), we found that they exhibited distinct responses to landmark manipulations. While landmark-tuned units decreased their response to omission or swap manipulations with a range of residual responses, non-landmark tuned units were either insensitive to landmark manipulation or increased their response to the omission of a landmark. Our results suggest that distinct populations encode the "visual" and "non-visual" components of the landmark representation, which together allow for decoding the location of the landmark, as well as whether it existed at that location previously.

A25 Odor plume navigation in the *Drosophila* central complex

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Flies integrate odor signals with wind direction signals to navigate towards food. Recent work from our lab has identified a pathway between two central brain regions, the Mushroom Body (MB) and the Central Complex (CX). This pathway encodes odor and promotes upwind navigation, a behavior necessary to find the odor source. We also have identified a group of CX local neurons called hΔC that integrate odor with wind direction information. However, how information is represented and transformed within this pathway during ongoing navigation is not known. I have developed a 2-photon compatible odor and wind-navigation paradigm that allows me to measure calcium activity during closed- and open-loop olfactory navigation. Using this paradigm, I have imaged neural activity at three mono-synaptically connected nodes along the pathway from the MB to the CX: (1) MB output neurons that drive upwind navigation, (2) an olfactory CX input called FB5AB, and (3) hΔC local neurons. I find a striking transformation of temporal information between FB5AB and hΔC neurons. While FB5AB responds transiently and is tightly locked to odor onset, hΔC exhibits slowly ramping and sustained activity that is more loosely coupled to the odor stimulus. During closed loop trials, hΔC exhibits a fairly stable bump of calcium activity that often appears during prolonged upwind runs. In contrast, open loop trials evoke stronger hΔC activity during wind and can cause the bump to rotate across columns. Taken together, our results suggest that hΔC activity encodes a conjunction of wind, odor, and locomotor/idiothetic variables to preferentially encode directed running during odor. Future experiments aim to examine the activity and functional role of this population during a more complex plume navigation task.

↑UP

A26 **Oriented evening flight behaviour in the Bogong moth revealed through automated video tracking**

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During their period of summer dormancy, Australian Bogong moths Agrotis infusa undertake seemingly random evening flights, filling the air with densities in the dozens per cubic metre. The purpose of these flights is unknown, but they may serve an important role in Bogong moth navigation, which remarkably enables them to return to the same exact summer sites—generation after generation—after migrating around 1000 km, and with no opportunity to learn their route or destination from prior generations. The recent development of the camera-based insect monitoring method, Camfi, enables quantitative observations of Bogong moth behaviour at an unprecedented scale. To gain a better understanding of the summer evening flights of Bogong moths, we have extended Camfi to facilitate automated video tracking of flying insects, taking the already-high throughput of the method to a new level. We used this new method to record the evening flight behaviour of Bogong moths in two elevational transects below the summit of Mt. Kosciuszko, NSW, on a single night in February 2021, and found that these flights were not random, but were systematically oriented in directions relative to the azimuth of the summit of the mountain. These results stimulate interesting and plausible hypotheses relating to previously unexplained summer evening flight behaviour of Bogong moths, and the mechanisms of their long-distance navigation.

A27 **Spatial representation in the hippocampal formation of barn owls with multiple flight goals**

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In a recent study, we found place-cells in the hippocampal formation of barn owls (*Tyto alba*) flying back and forth between two perches. Here we expand the research to address spatial representation in the hippocampal formation of owls when there are multiple flight goals. To achieve this, four perches were mounted, one on each wall of a flight room. A feeder was positioned adjacent to one of the perches. To obtain the food, the owl needs to fly between the remaining three perches until it finds the perch which standing on it opens the feeder's door. Every trial the perch associated with the feeder is switched to a different perch. Wireless tetrode recordings with custom-made microdrives were performed simultaneously with the behavior to collect the activity of single neurons in the hippocampal formation. As in our previous study, we found in the hippocampal formation cells with restricted place fields during flight. However, the place fields where mostly task related. For example, a neuron fired when the owl entered a place field when flying from the east perch to the west perch but did not fire when entering the same place field on the way to the north perch or on the way to the east perch. Some neurons fired when flying to land on a specific perch, no matter from where. In addition, some neurons preferentially fired when standing on one perch relative to the others. Finally, remapping occurred, in some place cells, when the owls switched tasks from flying between perches to avoid a negative reinforcement, to flying between perches to obtain a reward. Our results suggest that spatial representation in barn owls depends on the goals and behavioral context. The findings suggest similarities in spatial coding across mammals and birds.

A28 **Methods for the study of orientation and navigation in migratory bats**

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In contrast to established models in vertebrate navigation research, such as songbirds and salmonid fish, the study of mammals, i.e., their sensory physiology and neural mechanisms facilitating migration, have long been hampered by the lack of a correlate of their migratory orientation behaviour. Among bats, which became a model for short-range navigation in neuroethology quite early because of their echolocation capacity, a number

of species are also migratory, seasonally moving over >1,000 kilometres. These bats may qualify for neuroethological studies of migratory navigation mechanisms given the right test apparatuses and behavioural assays available. Here, we present such data from European migratory bats showing consistent orientation decisions across years, i.e., over many late summer migration seasons. Initially, we tested whether takeoff would be a meaningful orientational behaviour producing non-random directional data. Once this was established, we developed an easy-to-use apparatus (the circular release box for bats) which enables recording of takeoff orientation based on tracks a focal bat leaves on a thin layer of chalk. During an experiment, a bat is set free from a distance inside a circular arena, so that it can freely choose a preferred direction for takeoff. Importantly, this small scale-approach enables us to meticulously control environmental cues.Yet, the use of a cue in controlled conditions does not necessarily mean that the same bat would actually use this cue for navigation in free flight – when other cues may be available. Therefore, we have established that translocated, free-flying migratory bats equipped with radio transmitters (<1 gram) also show seasonally appropriate orientation responses. We provide a set of experiments as examples here.

A29 Modeling multiple context-specific vector memory in the insect central complex

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The insect central complex (CX) is a major centre for integration of multiple streams of sensory and/or memory information. As such it represents a key component of navigation capacity in many insects, from central-place foragers like bees or ants to *Drosophila*. A neural circuit contained in a part of the *Drosophila* CX, the ellipsoid body integrates multisensory sources to create a robust compass. The geometry of downstream neurons is suited to integrate this compass information with self-motion generated optic flow to create a vector memory of the insect's foraging path (path integration) and generate consistent homing behaviour. The CX circuits for retaining navigational vectors can be more generally used to guide goal-oriented behaviour that uses other input, like long-term memory output from the mushroom bodies (MB) or the innate attraction to single conspicuous cues. In addition, it has been proposed that some neuron sets allow vector rotation and allow cartesian representation or context-specific behaviour. Using computational modelling, we here integrate these new elements in an existing CX model to achieve both homing and goal-oriented behaviour. We propose that the co-existence of a positive (default) and negative (reversed) vector can underlie the integration of different sensory and memory related signals to create robust and context-specific navigation behaviour. Combined with biologically plausible synaptic plasticity rules, this CX model can transform a valence signal into a vector/direction as well as sustaining simultaneous memory of different vectors. Overall, our proposed circuit yields testable predictions about how visual-memory vectors based on MB output are combined with a path integrator.

A30 Harmonic radar tracking reveals unexpected effects of streetlights on moth orientation

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Moths are important components of almost all terrestrial food webs and play a crucial role as nocturnal pollinators. Recently, light pollution has been suggested as a possible driver of their decline, particularly because most species are strongly attracted to artificial light sources. However, so far it is neither sufficiently clarified when and why such "flight-to-light behaviour" occurs, nor whether orientation is affected in general. Using harmonic radar, we recorded trajectories of free-flying moths and linked these to the light environment quantified via all-sky photometry. We found that only 4% of individuals showed flight-to-light behaviour towards one of six streetlights that were evenly arranged in a circle around the release site. Nevertheless, the streetlights had a significant impact on flight behaviour as we observed a barrier effect on lappet moths (Lasiocampidae)

and an increased tortuosity of flights for lappet moths and hawk moths (Sphingidae). In addition, the moon as a natural light source played a key role in orientation performance. Our results show that the impact of light pollution on moths goes beyond flight-to-light behaviour, suggesting that its influence on orientation has been underestimated to date.

A31 A brainstem integrator for self-location memory and positional homeostasis

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To track and control self-location, animals integrate their movements through space. While self-location is represented in the hippocampal formation, it is unknown how such representations arise from integrated self-motion, whether they exist in more ancient brain regions, and by what pathways they control locomotion. Fish can be carried by water currents to potentially dangerous areas; here we report that larval zebrafish track their displacements to later return to previous locations. Whole-brain functional imaging revealed the circuit enabling this 'positional homeostasis'. A newly identified brainstem positional integrator stores a memory of past displacements and induces an error signal in the inferior olive, which controls future corrective swimming. Optogenetically manipulating functionally-identified integrator cells evokes displacement-memory behavior; ablating them, or downstream olivary cells, abolishes positional homeostasis. These results reveal a multiregional hindbrain circuit in vertebrates for integration of self-motion, memory of self-location, and control of locomotor behavior.

A32 Bumblebees navigate with vectors recalled from long term memory

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Path integration is a navigational strategy that allows an animal to maintain an internal estimate of its position relative to a point of origin. Hymenopteran insects are famous path integrators, directly returning to their hives after hundreds of meters of convoluted outward travel. We present that the bumblebee, *Bombus terrestris*, also uses path integration to locate their hidden hives after foraging while walking over short distances in an indoor circular arena. They estimate accurate home vector distances after displacement and orient their home vectors using an overhead polarization field. Walking bumblebees also exhibited systematic search patterns when home vectors failed to lead them accurately back to the nest, resembling searches performed by other species in natural conditions. Thus, our system provides a robust method to test navigation behavior in the laboratory that reflects major aspects of natural path integration. Interestingly, we also found that bees would orient their home vectors in respect to the orientation of the polarized stimulus learned from previous days of foraging. By experimentally dissociating vectors expected by short-term memory and long-term memory, we provide evidence that path integration-based home vectors were recalled from long-term memory when at a familiar feeder during our experiments. Further, bees would more often exhibit home vectors predicted by short-term path integration memory then vectors recalled from long-term memory once the long-term vector memories proved incorrect. This suggests that parallel vector memories are stored in the brain and that bees have access to both simultaneously. These data offer a potential mechanism for a vector-based analog of a cognitive map in insects.

A33 Ups and downs to visually gauge the flight distance

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During the waggle dance, bees communicate the direction and distance of a food source to their nest mates. Previous studies suggested that the odometer (distance-meter) of the foragers' path integrator gauges distance by integrating the raw angular velocity of the image sweeping backwards across their ventral viewfield, known as translational optic flow. The question arises as to how integration of optic flow (expressed in rad/sec) can reliably encode a distance, since it is dependent on flight speed and height. Bees and butterflies oscillate up and down as they fly forward at a frequency between 1Hz and 5Hz. Thus, insects enrich their ventral optic flow vector field by adding an expansion and contraction component: the optic flow divergence. Their current height of flight might be estimated by means of the optic flow divergence to scale the mathematical integration of the optic flow to gauge the distance travelled. In Bergantin et al (2021), we developed a self-scaled model of the bee's visual odometer (called SOFIa). Our simulation included the bouncing trajectory of a bee under various wind and terrain conditions. Our findings indicate that the visual odometer is particularly robust to wind and relief regardless of the ground height and speed: the statistical dispersion of estimated flight distances was reduced 10-fold compared to that obtained without scaling. We tested the SOFIa model by equipping a hexarotor with four optic flow sensors and fusing their outputs to measure the translational and divergence optic flow cues. Our results show again the accuracy of this visual odometry strategy (between -2.6% and 2.7% during 30m flights). Such approach is particularly interesting for robotic applications based on minimalistic visual equipment in GPS-denied environments.

A34 Learning flights in bumblebees

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When leaving an important location, bumblebees, like honeybees and wasps, perform elaborate learning flights. They acquire visual information that guides them on their return. We have previously shown that learning flights vary when set in the context of the nest or a flower, in ways that reflect their functional significance. The nest is unique, in a permanent location and inconspicuous. Bees invest more time and effort in memorising the nest's location during a learning flight than that of a flower which is a transient resource and conspicuously coloured. The experience of a flower's reward also modulates a bumblebee's learning flight but so far, we had presented bumblebees only with a single flower. It is known from their natural foraging behaviour that the decision to return to a flower is influenced by other flowers growing in its immediate surroundings. We therefore recorded learning flights in bumblebees that fed and departed from a highly-rewarding flower located in a patch of either two or five artificial flowers. All flowers were of the same size and colour. Our preliminary analysis and comparison with learning flights from a single flower indicates that bees frequently turned back to look at the flower they departed from. However, their their viewing directions were more variable. When they returned, bees were not more likely to visit the flower they had previously fed from. This suggests that bees might memorise the whole patch as a valuable location after associating the flower's colour with a high reward and detecting other flowers nearby during the learning flight.

A35 What are the rules of spatial learning? Insights from ant navigators

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Desert ants are excellent navigators. Each individual learns long routes meandering between the trees and bushes of their natural habitat. We know well how the insect brain can memorise and recognise views, and how this recognition enables them to find their way. However, practically nothing is known about how learning is orchestrated in the first place. What are the rules that guide spatial learning? Learning a route cannot be governed only by rewards and punishments, but may happen '*continuously*'; a vague concept called 'latent learning'. Our results in ants, freshly out of the oven (the hot desert field), surprised us, as they point at a remarkably sophisticated, yet bizarrely rigid, way of learning.

A36 Neural representation of head-direction across brain areas in quails

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In mammals, head-direction tuned cells (HD cells) are thought to form the basis for the sense of direction, which is crucial for navigation, orientation, and spatial memory. We recently discovered HD cells in the hippocampal formation of an avian species - the Japanese quail (Coturnix japonica). To further explore the direction coding system of birds, we applied a state-of-the-art high-density recording technique (Neuropixels) in freely behaving quails. Deep learning-based video processing algorithm (DeepLabCut) was used to automatically track the quail's body position and head direction throughout the recording session. We analyzed the activity of over 5000 single units in nine quails. We first performed single cell analysis and determined the distribution and tuning curves of head direction cells in different brain regions. Surprisingly, we found HD cells in a variety of brain regions, including the hippocampal formation, hyperpallium, mesopallium, nidopallium and the dorsal thalamus – but HD cells were most abundant in the hippocampal formation and the dorsal hyperpallium, where they also had the sharpest tuning. Next, we trained several decoders, both statistical models- and machine learning-based to reconstruct the quail's head direction from the neural data of cells ensembles recorded in each region. Our analysis show that neural population responses in the hippocampus as well as a number of other brain areas hold precise information about head-direction. Taken together, this study provides the first systemic characterization of the bird's head-direction coding system across brain areas. Our data reveal that stable head-direction representation expands beyond the boundaries of the avian hippocampus homologue.

A37 Finding the upper bound of disruptive radio frequencies disrupting avian magnetoreception with behavioural experiments

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During their biannual migration, night-migratory songbirds can use magnetic compass besides other mechanism to guide their way. This magnetic compass is known to be a light-dependent inclination compass. The magnetic sensor of this compass is most likely two molecules that get excited into radical states, where the geomagnetic field can take effect. This quantum chemical process is the so-called radical-pair mechanism and the involved molecules are believed to be a flavin-tryptophan radical pair. Broadband radio frequencies (RF) fields are known to disrupt this magnetic sensor more than single frequencies, and have been used to determine how long the radical pair can be affected by the geomagnetic field. Whether certain RF field can affect the mechanism is depending on the number of magnetic nuclei (hydrogen and nitrogen atoms) in the radicals and the hyperfine interactions these produce. Based on the assumption of a flavin-tryptophan based sensor, quantum chemistry predicts non- or disruptive RF broadband fields, which would identify certain aspects of the radicals. Guided by these predictions, we tested the magnetic compass orientation behaviour of night-migratory songbirds under two RF field conditions. RF fields of 75-85 MHz had a disruptive effect on the orientation behaviour and indicated that at least one radical has as many hyperfine interactions as a flavin-tryptophan radical pair would. With RF fields of 235-245 MHz, an upper bound of RF fields affecting the radical-pair mechanism was set, since the applied fields did not show any effect on the birds' magnetic orientation behaviour. These findings are consistent with predictions for bio-organic molecules and show that the upper cut-off of disruptive RF fields lies somewhere between 75 and 235 MHz.

A38 The head direction circuit of ants and bees

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A fundamental feature of animals is their ability to navigate. In each moment in time the animal's brain has to compute the current heading of their body in space, which then can be compared against a goal direction to drive steering movements. The neural circuits that use sensory information to encode heading are well studied in the fruit fly. In principle, external sensory information and rotational self-motion are integrated in the central complex (CX) to generate a single bump of neural activity within a ring-shaped neural circuit. Components of this intricate system have been revealed in many insects and volume electron microscopy in bumblebees has confirmed that key neural projection patterns are indeed highly conserved. Yet, computational modeling has shown that even small differences in cellular morphology can have profound effects on circuit performance. We thus asked how head direction circuits differ in species that pursue diverse navigational strategies in distinct sensory environments. Based on block-face electron microscopical data we analyzed these circuits in five species of bees and ants. While the core head direction circuit is remarkably conserved, its inputs from sensory areas vary to a much larger degree. Our data suggest that the relative contribution of parallel input pathways depends strongly on the information available in the habitat of a species. Furthermore, despite general conservation of the core circuit, we found numerous features of individual components that differed between species, in particular in those circuit elements required to transform the linear anatomical arrangement of neurons into a ring-shaped topology. In this respect, even comparably closely related species have evolved different solutions to the same problem.

A39 Neural circuit dynamics for navigation and sleep observed over multiple days in behaving fruit flies

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The impact of sleep on neural circuits is only partially understood. In the brain of Drosophila, circuits for sleep control as well as circuits for navigation and memory have been identified in the central complex, an area located in the center of the fly brain. This brain area offers an opportunity to understand the impact of sleep on navigation related circuits at the level of small populations of identified cells with known connectivity. To describe the coupled dynamics of sleep and navigation circuits in the central complex, we have developed a computational model that we investigate analytically as well as numerically. The model suggests a function for sleep in the navigation (head-direction) circuits of the fly [Flores-Valle et al., Plos. Comp. Biol., 2021]. To test predictions of this model experimentally, we have developed an automated in vivo two-photon imaging method for monitoring behavior and neural activity in head-fixed walking flies during sleep and wakefulness for up to one week [Flores-Valle et al., J. Neurosci. Meth., 2021]. To investigate to relation between sleep and memory, we have additionally developed a novel spatial learning paradigm for tethered walking flies during functional imaging [Flores-Valle et al., submitted]. Here, we use these methods to describe calcium dynamics in different cell populations important for navigation, sleep and learning over multiple days and nights. Using this approach, we quantitatively describe for the first time the dynamics of a sleep homeostat - a neural circuit measuring the time spent awake or sleeping - in a behaving animal.

A40 Conserved parallel input pathways to the noduli across hymenopteran insects

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All insect species share a group of neuropils at the center of the brain called the central complex, which integrates sensory modalities with the internal state to compute motor output. Amongst these neuropils, the noduli stand out as a hub for sensory input related to self-motion. In *Drosophila*, the noduli have five layers coinciding with pathways of sensory input (via LNO cells) and specific types of PFN cells. In contrast, bumblebee noduli showed no layered organization, in theory allowing connectivity between pathways. Local circuits were posited by models of path integration, an ability highly developed in bees and ants, we asked if a simplified noduli outline is common in hymenopteran insects. We used block-face electron microscopy to produce image stacks of the central complex of species of bees and ants. We combined manual neuron tracing (via CATMAID) at medium resolution with automatic segmentation and synapse annotation at high resolution to reconstruct PFN and LNO cells in the noduli. We found three regions across the studied species: the main (NOm) and small units (NOs) receive input from LNO cells, the cap (NOc) receives input from tangential cells of the fan-shaped body. All regions are targeted by columnar cells: PEN (NOs), PFN (NOm) and PFNc (NOc). While regions are similar across species, their relative volumes, and the numbers and projection fields of LNO and PFN cells were species specific, suggesting different input pathways. Despite their overlap, connectivity between bee PFN and LNO cells showed three parallel pathways within the NOm. This highlights that connectivity analysis can reveal precise circuits in seemingly unstructured brain regions. Furthermore, it suggests that parallel self-motion input pathways are conserved between flies and bees.

A41 Cross-species comparison of mammalian spatial planning using naturalistic predator-prey interactions

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The ability of mammals to imagine multiple future states and select sequences of actions to achieve a desired state—known as planning—is a fundamental aspect of behavior in highly dynamic conditions. It allows mammals to perform feats such as using obstacles to either stalk prey or avoid predators. Our lab previously performed predator-prey simulations to investigate the evolutionary basis of planning, providing evidence of planning's dependence on the visual range of an animal and the configuration of obstacles within their environment. For empirical testing of these computational results, we have developed a new mouse experimental paradigm that provides more ecologically relevant testing conditions than other paradigms such as T-mazes or two-step tasks. Water restricted mice traverse a reconfigurable hexagonal arena to reach a water reward while avoiding an aggressive autonomous robot "predator" using air puffs as an aversive stimulus. While we used standard *Mus musculus* (C57BL/6) mice to test the impact of the environment on their planning, we have also tested two additional mice species with higher visual acuity in contrast to the reportedly poor vision of C57BL/6 mice: *Peromyscus maniculatus* and carnivorous *Onychomys torridus*. We have observed evidence of planning behavioral markers such as pausing, peeking, and rerouting of their trajectories. This behavior is most evident in environments favorable for planning, and we have identified similar patterns across all mice species despite their large evolutionary distance. These results lend support to the prior computation findings on planning's environmental dependency and support the conservation of planning behavior across mammals, laying the foundation for continued work using these naturalistic conditions.

A42 Comparison of motivational dynamics of local search behaviour and honey bee dance

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When a hungry fly finds a sugar drop, it starts a local search around it. Dethier was the first to describe this behaviour in blow flies and compared it to the honey bee dance. He showed that the initiation of the search is not dependent on the location of the food, similar to how dance is started in the colony away from the food

location. We did a passive displacement experiment behaviour using fruit fly (*Drosophila melanogaster)* and the Western honey bee (*Apis mellifera)*. Flies were giver sugar and before they started walking, they were transferred it to a plate with no food. Honey bee foragers, trained to an artificial feeder were tested similarly, inside the lab. Both flies and bees start a search around the point where the animal is released, and not where it had been fed. This shows that sugar intake does not directly start the search, but increases the motivation to initiate a search. To understand how long is the time window for this motivation, the flies and bees were not allowed to walk, post-feeding for different time periods and then released. The heightened motivation to initiate search lasts for 3 minutes for flies and 5 minutes for bees. We are now asking the question: when a successful honey bee forager is delayed from going back to the hive, does it affect its motivation to dance? Foragers are captured at the feeder after collecting sugar, for different time periods and then their dance is observed. We hypothesize that in both behaviours, search and dance, sugar intake induces comparable changes in the same neurotransmitter/neuromodulators. This will be tested with a mass spectrometry of individual flies and honey bee brains. Such a comparative approach will provide a deeper understanding of neural mechanisms underlying search and dance behaviour.

A43 Tracking the orientation and 3d path of flying insects

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Tracking the location and orientation of insects in the landscape at the meso-scale [<40m] gives us access to pose/location data key to learning and cognitive modelling, for example revealing where insects are looking. It allows exploration of the responses to landscape changes and enables researchers to learn about the ontogeny of features such as learning flights or foraging behaviour. For other researchers, being able to automate the monitoring of pollinator behaviour (tracking which flowers are visited more / less in a constrained greenhouse or similar) allows the scaling of experiments to answer more questions. Our previous work performed real-time tracking of insects in the field using retroreflective tags and a system of cameras and flashes. These have since been improved to make them more robust and easy to use. In our latest work we have added three important components to widen their range of applications:

- 3d (probabilistic) flight path reconstruction.
- Individually identifiable tags: Using colour retroreflectors.
- Inferring orientation: Using multi-colour tags that allow the orientation of the insect to be inferred from the tag colour.

We demonstrate the system by recording the learning flights of bumblebees (*Bombus terrestris*). We start, using this data, to explore the similarities and differences between bees. We explore how successive learning flights evolve. Earlier research found bees face predominantly towards the nest during the early (micro-scale) stages of the learning flight. We use our system to see how this behaviour changes at this meso-scale. In summary, this system provides a low-cost platform for tracking behaviour at the meso-scale: opening up a window on to behaviour largely hidden from us.

A44 Too cool to remember

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Solitary foraging insects use path integration to estimate the direction and distance to their nest. This estimate, the home vector, is updated continuously and is reset once an ant enters its nest. Ants prevented from returning to their nest remember their home vector for several hours. The conjunction of fast update and long persistence of the home vector does not directly map to current short, mid, and long-term memory accounts. We sought to investigate the nature of this memory using cold-induced anaesthesia (chill-coma). We hypothesised that chill-coma anaesthesia would disrupt any reverberating activity in recurrent circuitry, which has been hypothesised to underlie home vector memory. We captured *Myrmecia croslandi* foragers 11m away from their nest ('full vector') and returning foragers outside their nest ('zero vector'). We kept all ants in darkness for 30min, half of them at ambient temperature and the other half at 0ºC, inducing chill-coma. Once the anaesthetised ants regained locomotion, we gave food to all ants and released them individually in an unfamiliar location. Zero vector ants searched for their nest while non-anaesthetised full vector ants ran towards the fictive nest location. By contrast, anaesthetised full vector ants walked in the fictive nest direction but did not walk as far as the non-anaesthetised full vector ants. Using modelling, we found that the best explanation for this phenomenon is that the home vector is stored in Cartesian coordinates and that chill-coma proportionally reduced the memory across all coordinate axes by around 85%, resulting in degradation of the distance but not the direction memory. Our results pose strict constraints on the plausible biophysical mechanisms that may underpin path integration memory.

A45 An unusual lateral protocerebrum in larval mantis shrimps

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Mantis shrimps, more formally known as stomatopod crustaceans, are predatory marine arthropods renowned for their highly developed sensory systems and devastating raptorial strikes. Their visual systems are unrivalled in complexity, being capable of color vision, linear and circular polarization vision, and motion vision. Mantis shrimp are known to orient and navigate using celestial cues, and are the first fully aquatic animals described to exhibit path integration. In insects, the neurobiological underpinnings of these behaviors are attributed to the central complex (CX) and lateral complex (LX), and in fact the neural organization of both these brain regions in mantis shrimp closely resemble those found in insects. Like many insects, developing stomatopods metamorphose through a series of larval stages that are ecologically, sensorially, and behaviorally distinct from the adults. Although developing mantis shrimp possess many of the principal neuropils described in adult mantis shrimp, including the CX and LX neuropils, their overall neuroarchitecture is markedly different. Here, we discuss the central brain neuroarchitecture of the larvae of the mantis shrimp *Neogonodactylus oerstedii*, focusing in particular on the unusual larval lateral protocerebrum.

A46 Modelling gap choice through cluttered environments in pigeons

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Animals are frequently faced with the challenge of negotiating unfamiliar structured environments during goal-oriented behaviours. Recent work on visually-guided flight through clutter has conceptualised this as a process of identifying and negotiating gaps that are broadly aligned with the animal's expected or intended goal. These gaps are thereby conceived as attractors in their own right, rather than as features incidental to obstacles acting as repellers. Here we ask what information birds use to detect and fly through such gaps by tracking pigeons flying through an artificial forest of vertical poles. We find evidence for pigeons combining brightness cues with the sizes of visual gaps available ahead to choose which clearances to fly through. Modelling the free-flight behaviour as a process of sequential gap selection allows us to decompose it into components that can be independently evaluated. We find the observed behaviour is best captured by an autoregressive model including the effect of brightness, the absolute distance between obstacles and apparent visual gaps between a bird's position and the end of the obstacle field. This can, in turn, be combined with established models of pigeon steering to provide a more complete picture of how birds guide their flight through complex environments.

MOTOR SYSTEMS, SENSORIMOTOR INTEGRATION, AND BEHAVIOR II

B12 Tardigaits: coordination and neuromodulation of tardigrade locomotion

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Animals move deliberately through their environment, and much of their body plan and physiology are dedicated to this activity. Tardigrades, commonly known as “water bears,” are 3 mm long invertebrates that use eight, short, clawed legs for locomotion. Moreover, studying tardigrade locomotion enriches our pool of examples for animal locomotion, because they are their own ancient phylum. We recorded the locomotive patterns of an aquatic and a semi-terrestrial species of tardigrades while swimming and walking by video microscopy and kinematic analysis. We found that that tardigrade locomotion patterns were variable and cannot be described adequately described by identifying stereotypic gaits, such as trot, hop, or bound, which have been the focus of studies that describe locomotion of limbed animals. Only a small fraction of tardigrade locomotive behavior is captured by these 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, while swimming produces front-to-back propagation of leg motion on each side with motionless hind legs. 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 induced 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 be used to inspire stable multi-legged robots and prompt further research on tardigrades to determine the neural basis of their locomotion.

B13 Sublethal effects of the pesticide Flupyradifurone on locomotion and behavior of *Chrysoperla carnea* larvae

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In recent decades, the scientific society has observed a great loss of insects. Several factors have been identified as the main cause of insect declines especially the use of insecticides. It is already proven that these substances do not only kill pests but also have an impact on the survival of beneficial insects. A relatively new pesticide is Sivanto whose active ingredient is Flupyradifurone. Unfortunately, such substances are often tested for bees, but other non-target organisms are not considered. For proper risk assessment and to counteract further insect decline, it is necessary to study the effects of these pesticides in a variety of species at sublethal concentrations. Thus, we investigated the sublethal effects of Flupyradifurone on the mobility and behavior of *Chrysoperla carnea* larvae. Our results showed the occurrence of abnormal behaviors such as trembling and comatose animals even at the lowest doses. In addition, longer video-based observations combined with computer-aided tracking could confirm that the behavior of the treated animals differed from that of the control at all concentrations. In the short term, hypoactivity was observed, which sometimes changed to a hyperactive state as time progressed. High-speed analyses were used to investigate the effects of Flupyradifurone on larval locomotion. For a short time, the effects were very large, so that the animals were no longer able to move. Over time, however, many animals recovered and showed no effects on their locomotion. Despite its declaration as “bee-safe”, Flupyradifurone showed strong sublethal effects on beneficial insects, such as *C. carnea*. For future risk assessments, it is therefore essential to use different species and sub-lethal concentrations to obtain a realistic picture.

↑UP

B14 Proprioceptive body-state feedback modulates visual object tracking in *D. melanogaster* flight

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Animals operate in complex sensory environments requiring integration of moment-to-moment feedback across multiple sensory modalities. During flight, reflexive stabilization of visual gaze and voluntary object tracking occur in parallel visual processing streams. The processes control smooth optomotor movements to stabilize the visual panorama and whole-body saccades that allow the animal to rapidly orient toward features. Much of our understanding about integration of gaze stabilization and feature-detection comes from fixed tether paradigms which disable real proprioceptive feedback. While the visual feedback loop can be artificially closed, the animal cannot move and information about body state is highly restricted. We use a magnetic tether paradigm where the body is freely rotating in one plane, allowing for closed-loop behavioral control of both visual and proprioceptive sensory signals. We show that whereas body-fixed flies robustly orient toward and smoothly track movements of a vertical bar, these responses are weak or completely absent in the presence of proprioceptive feedback. We hypothesized that proprioceptive signals modulate how object motion is processed. To test this, we designed a pneumatic gripper that allows switching between body-fixed and body-free state within a fly, flexibly opening and closing the proprioceptive feedback loop. We show that body-fixing increases the gain of smooth optomotor steering responses to bar motion on midline, suggesting a change in the balance between optomotor gaze stabilization and object-tracking. Ongoing genetic perturbations are testing whether the mechanism responsible involves feedback from the gyroscopic haltere sensorimotor system and will take us closer to understanding multisensory flight control.

B15 The multiple locomotion gaits of the mole cricket

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The European mole cricket (*Gryllotalpa gryllotalpa*) is a subterranean nocturnal insect and among the few insects known to possess fossorial (digging) legs. Enlarged dactyl claws on the insects' tibia enable their unique soil-dwelling, subterranean existence. Other modifications related to life in an underground network of tunnels comprise their enlarged, thrust-providing hind legs and cylindrical body. The insect's subterranean locomotion behavior presents very rapid forward and backward walking. There is relatively little information available, however, regarding the locomotion gaits in this unique insect. Using a custom-built tubular, transparent set-up, high-speed video tracking, and cutting-edge off-line analysis tools, we acquired a comparative description of the kinematics of forward vs. backward walking in adult mole crickets. Highlighting the form-function relation of the different legs, we provide a detailed description of the spatio-temporal aspects of all leg movements during double-tripod forward walking. We further identify two backward locomotion gaits: (1) In response to aversive stimuli, the insect demonstrates several (up to ten) cycles of a never before described backward galloping gait. This is characterized by left-right synchronization of the middle and hind legs, while the front legs alternate. (2) Following this unique gait, the insect switches to a different backward gait with double-tripod phase relations. This is not merely a "rewind playback" of the forward walking, as the different legs demonstrate different kinematics. We are currently complementing our video analyses with simultaneous electrophysiological recordings from the leg muscles during locomotion to provide a complete picture of the different locomotion gaits of the mole cricket.

B16 Escape behavior in zebra finches (*Taeniopygia guttata*) and the role of the isthmotectal system

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Avoidance reactions to looming objects are prominent behaviors in all vertebrates. In birds, wide-field tectal ganglion cells (TGCs) and their thalamic target in the nucleus rotundus (RT), forming the tectofugal pathway, are strongly responsive to looming stimulation. To investigate the escape behavior in birds and its dependence on the tectofugal pathway, we first tested the behavior of zebra finches to an expanding disc presented in different parts of the visual field. Then, we assessed these responses after performing unilateral kainic acid lesions in the parvocellular isthmic nucleus, which gates retinal signal transmission from the TGCs to the Rt in the ipsilateral tectum (TeO). We found that adults responded with ducking, running or flying to looming stimulation, being the last two the more frequent responses. The probability of responses increased when the stimulus was delivered in either the upper or frontal binocular fields compared to monocular stimulation. These avoidance maneuvers began to appear between 5-7 days after fledging. On the other hand, isthmic lesions completely abolished avoidance behavior triggered from the contralateral eye whereas that from the ipsilateral eye remained intact. Other behaviors, like pecking, perching, and aggressive biting were clearly elicited from either eye. Our results show that escape behavior in zebra finches has delayed development and is highly sensitive to stimuli approaching from above, as demonstrated in rodents and other species, although in zebras frontal binocular stimulation was similarly effective. Finally, the lesion results suggest that isthmic input to TeO is especially determinant in the tectal transmission of retinal signals leading to escape reactions, sparing other visuomotor maneuvers.

B17 Facial movements and their neural correlates reveal latent decision variables in mice

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Signals related to self generated movements are broadcast to non-motor brain regions, and can be detected across wide regions of the forebrain in mice. Indeed, it has been observed that, even during the performance of a task, incidental movements, or 'fidgets', account for much more variance in neural activity than task-related movements and task-related variables. Some bodily movements or expressions, such as those of the face, reflect biologically significant expressions of internal state, that is, emotions, which have important relationships with ongoing cognitive processes such as decision-making. Therefore, it is possible that movements characterized as incidental actually express latent internal states that are in fact related to decision processes. Here, we trained mice to perform a probabilistic foraging task while video monitoring facial movements and simultaneously recording large ensembles of neurons in frontal and premotor cortical regions using Neuropixels probes. In this task, mice had to combine a sequence of successful and failed foraging attempts to compute a latent decision variable (DV) in order to time the duration of their foraging bout. After training, this DV predicted the mice's behavior and accounted for a large degree of premotor activity, but consistent with a previous report, high dimensional facial movements dominated neural activity in premotor cortex and in all the other recorded brain regions. Remarkably, however, further analysis revealed that the explanatory power of facial movement was largely due to its correlation with the latent DV. The component of the movement uncorrelated with the latent DV had little predictive power. The premotor representation of the DV temporally preceded that of the movements, suggesting that premotor activity contributed to generating facial movements rather than reflecting proprioceptive feedback. These results show that seemingly 'incidental' bodily expressions can in fact reveal otherwise hidden task-relevant states.

B18 Threat history controls escape behaviour in mice

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In many instances, external sensory-evoked neuronal activity is used by the brain to select the most appropriate behavioural response. Predator avoidance behaviours such as freezing and escape (de Franceschi et al., 2016; Yilmaz & Meister, 2013) are of particular interest since these stimulus-evoked responses are behavioural

manifestations of a decision making process that is fundamental to survival (Evans et al., 2018; Vale et al., 2017). Over the lifespan of an individual however, the threat value of agents in the environment is believed to undergo constant revision (Cooper & Blumstein, 2015) and in some cases repeated avoidance of certain stimuli may no longer be an optimal behavioural strategy (Rossier et al., 2021). To begin to study this type of adaptive control of decision making we devised an experimental paradigm to probe the properties of threat escape in the laboratory mouse, Mus musculus. First, we found that while robust escape to visual looming stimuli can be observed after two days of social isolation, mice can also rapidly learn that such stimuli are non-threatening. This learned suppression of escape (LSE) is extremely robust and can persist for weeks and is not a generalised adaptation since flight responses to novel live prey and auditory threat stimuli in the same environmental context were maintained. We also show that LSE cannot be explained by trial number or a simple form of stimulus desensitisation since it was dependent on threat-escape history. We propose that the action selection process mediating escape behaviour is constantly updated by recent threat history and that LSE can be used as a robust model system to understand the neurophysiological mechanisms underlying experience dependent decision making.

B19 Blink and you'll miss it: ballistic predatory behavior in the ogre-faced spider

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Nocturnal animals, who live in a world dramatically darker than their diurnal relatives, typically possess sensory systems attuned to their dimly lit lifestyle. The ogre-faced spider, *Deinopis spinosa*, is strictly nocturnal and possesses sensory systems well-adapted for nocturnal foraging – massive, motion-sensing eyes, complemented by multiple sensory organs used to detect airborne acoustic information. These spiders also exhibit a unique foraging strategy, termed "net-casting," where individuals suspend themselves above a substrate with outstretched front legs holding a rectangular, fuzzy net. Using this net, spiders actively ensnare prey walking beneath or flying above them. While vision is useful in capturing prey from off the ground, spiders use the acoustic cues of flying prey to snatch insects from out of the air. This "backward strike" towards aerial prey happens in the blink of an eye and requires the coordination of all eight legs to successfully capture insects zipping past. Little is known of this ballistic predatory behavior. Here, through the use of high-speed video and machine learning software, we illuminate the directionality, accuracy, and kinematics of this fascinating and tremendously impressive behavior.

B20 A comparative analysis of vestibular-motor behaviors in bats and mice: insights into species-specific sensorimotor functions in the mammalian brain

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The vestibular system detects head motion to maintain gaze and postural stability. Differences in locomotive repertories across species could produce strong evolutionary selection on the vestibular system. Compared to terrestrial animals, aerial mammals such as bats, place unusual demands on their vestibular systems as they negotiate 3D space in flight. Thus, given the different locomotor challenges of terrestrial and aerial mammals, we hypothesized that there are species-specific differences in vestibular processes. To test the hypothesis, we studied vestibular functions in Seba's short-tailed bat, which uses vision and echolocation to navigate and find fruit in its environment. Vestibular and visually evoked eye movements were recorded using an infrared video system (ISCAN). Specifically, we quantified semicircular canal and otolith function by measuring rotational vestibulo-ocular reflex (VOR) and off-vertical axis rotation (OVAR) response, respectively. To assess visual function, optokinetic reflex (OKR) responses were quantified. Stimuli comprised sinusoidal rotations of (16 deg/s; 0.2-2Hz) and step stimuli (50 deg/s). Comparisons were made with eye movements recorded in C57BL/6 mice in these same conditions. Surprisingly we found that unlike mice, bats demonstrated negligible eye movement responses to semicircular canal activation during aVOR. Yet, like mice, the bats generated robust eye movements to otolith stimulation during OVAR and visual stimulation during OKR. Thus, our results - reporting for the first time the vestibular/visual responses of Seba's short-tailed bats - suggest a reliance on otolith over semicircular canals for gaze stabilization, consistent with species-specific differences in vestibular processing.

B21 Integration rules for multisensory control of wing and gaze revealed by direct haltere manipulation

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The true flies (order Diptera) possess unique sensory organs known as halteres. Necessary for flight, the halteres are a set of reduced hindwings that sense inertial forces arising from body rotations *via* an array of mechanosensory neurons called campaniform sensilla [1]. These specialized afferents form mono- and disynaptic connections with head and wing motoneurons, thereby mediating a set of stabilizing equilibrium reflexes. Many questions remain as to how this system represents information and how it acts in concert with descending visual inputs to command a shared pool of motoneurons (rev'd [2]). Here, we explore several aspects of haltere mechanosensation by driving movements of the halteres of the vinegar fly *Drosophila melanogaster* using a custom electromagnetic flight arena. In most species (including *Drosophila*) the halteres beat with an antiphase synchrony to the forewings. By disrupting this synchrony, we impair the fly's ability to precisely control the wingstroke amplitude and reduce the amplitude of head and wing responses to visual stimuli. In contrast, by preserving wingstroke synchrony but subtly altering the haltere's stroke path, we repeatably induce steering changes in the head and wings. When presented with a concurrent visual stimulus, we show that wing motor output reflects the sum of unisensory responses whereas multisensory head responses primarily reflect the influence of the visual system [3]. Finally, we show that genetic ablation of a specific class of thoracic interneurons labelled by the gene *Even-skipped* [4] results in flies that respond differently to haltere stimulation.

[1] Pringle (1948) Proc. Roy. Soc. B
[2] Yarger and Fox (2016) Int. Comp. Biol.
[3] Rauscher and Fox (2021) Proc. Roy. Soc. B
[4] Heckscher et. al. (2015) Neuron

B22 Mapping the sensorimotor connectome underlying protein-specific appetites in *Drosophila melanogaster*

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Dietary amino acids are key determinants of lifespan and fecundity. Flies achieve a balanced intake of amino acids under diverse physiological conditions by developing a protein-specific appetite. The Drosophila feeding motor program comprises behavioral modules (feeding bursts and activity bouts) which are tightly regulated by physiological states through feedback (e.g. nutrient deprivation) and feedforward (e.g. mating) mechanisms. Here, we aim to understand the neural-circuit mechanisms underlying physiological state-specific regulation of food intake by dissecting the functional and temporal relationships between protein deprivation and feeding as well as the underlying neural circuits. We discovered that there are temporal differences between regulating the frequency and the duration of feeding bursts, suggesting that distinct neuromodulatory mechanisms modulate these parameters. Different sensory neuron populations control frequency and duration of feeding bursts suggesting that distinct sensorimotor pathways regulate these feeding motor programs. We combined connectomics and trans-synaptic labeling to map neurons downstream of a group of gustatory receptor neurons (taste peg GRNs) that are important for sustaining feeding on proteinaceous food. High-resolution behavioral analysis and optogenetics confirmed the identity of a group of interneurons that specifically regulate the duration of feeding bursts in a nutrient-specific manner, suggesting different aspects of the feeding motor program are controlled by separate circuits.

B23 Visuo-motor control of locomotion in navigating ants

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Navigating ants regularly alternate between turning right and turning left when walking. Those oscillations are produced endogenously by an intrinsic neural oscillator, likely located in pre-motor areas, but can be modulated by sensory signals such as learnt visual cues to enable the ant to adjust its movements to the current situation. Here we investigated whether and how spontaneous visual cues such as optic flow influence ants' oscillations. We mounted *Cataglyphis velox* ants within a virtual reality set-up that enables to decouple the ant's motor movements from the optic flow perceived. We show that rotational optic flow strongly impacts the amplitude and frequency of the oscillations. By manipulating the sensory-motor relationship in various ways, as well as by covering one of the ants' eyes, we managed to form a simple neural model of how signals from both eyes are integrated and modulate the intrinsic oscillator. Notably, we show how the optomotor response emerges as a result of this modulation. In addition, our result implies the existence of bilateral efference copies that cancel the optic-flow signal generated by the ant's movement. Interestingly, these internal predictions become silent if the eye perceives no light source. Overall, our findings explain how oscillations are continuously and robustly regulated by optic-flow to produce an effective trajectory when in closed-loop with its environment.

EVOLUTION AND DEVELOPMENT II

C7 Social brain evolution of halitid bees

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A major transition in biological evolution was the shift from solitary to social living. In this transition the emergence of cooperative behaviors formed from the interaction of coordinating activities in groups. These behavioral adaptations are likely underscored by neurological pathways in the brain that are driven by the neurochemistry and/or physiological in social individuals and these neurobiological changes should differ from solitary organisms. The Sweat bees (Halictidae) presents a unique study groups, in which all the major transitions from solitary to eusocial species are present. Here we present a collection an ongoing research to elucidate the neuronal and behavioral adaptations to social living. We present data on four species across Halictid bees, *Nomia melanderi* (solidatry – communal living), *Megalopta genalis* (facultative eusocial), *Augochlorella aurata* (primitively eusocial) and *Augochlora pura* (solitary – ancestrally eusocial). We found that brain size tends to increase with increase sociality and is followed with a evolutionary loss of brain size with a loss of sociality. We also show that social influences can also reduce mushroom body size. We discuss the implication of how brains may have evolved to accommodate increase levels of sociality.

C8 Evolution of the olfactory circuits driving human host preference in mosquitoes

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The evolution of brain circuits during behavioral adaptation is both fascinating and widely unexplored. Its investigation requires related animals with divergent behavior as well as an advanced toolkit accessing anatomy and physiology of their brains. A derived subspecies of the yellow fever mosquito *Aedes aegypti* has relatively recently specialized to bite humans, while the ancestral subspecies bites a variety of animals. A recent study in human specialist mosquitoes revealed that the detection of human body odor relies on the activation of few key glomeruli that are together capable of driving strong attraction. However it remains unclear how host odor blends are encoded in ancestral generalist mosquitoes and how this glomerular code evolved. We introgressed the calcium indicator GCaMP6f expressed in olfactory sensory neurons into an animal-preferring strain of *Ae. aegypti* and used two-photon calcium imaging to investigate responses to relevant host odor blends in their antennal lobes. Comparison between odor-evoked activity in animal- and human-preferring mosquitoes is giving us our first insights into how host-odor coding has evolved in this mosquito's brain and led to it ultimately becoming a major global health threat.

C9 EyeVolve, a modular PYTHON-based model for simulating eye type diversification

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Vision is among the oldest and arguably most important sensory modalities for animals to interact with their external environment. Although many different eye types exist within the animal kingdom, mounting evidence indicates that the genetic networks required for visual system formation and function are relatively well conserved between speciescommonly deployed. This raises the question as to how common developmental programs are modified in functionally different eye types. Here, we approached this issue through EyeVolve, an open-source PYTHON-based model that recapitulates eye development based on developmental principles originally identified in *Drosophila melanogaster*. Proof-of-principle experiments showed that this program’s animated timeline successfully simulates early eye tissue expansion, neurogenesis, and pigment cell formation, sequentially transitioning from a disorganized pool of progenitor cells to a highly organized lattice of photoreceptor clusters wrapped with support cells. Further, tweaking just five parameters (precursor pool size, founder cell distance and placement from edge, photoreceptor subtype number, and cell death decisions) predicted a multitude of visual system layouts, reminiscent of the varied eye types found in larval and adult arthropods. This suggests that there are universal underlying mechanisms that can explain much of the existing arthropod eye diversity. Thus, EyeVolve sheds light on common principles of eye development and provides a new computational system for generating specific testable predictions about how development gives rise to diverse visual systems from a commonly specified neuroepithelial ground plan.

C10 Opsin evolution in jumping spiders

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Jumping spiders (Salticidae) often exhibit elaborate courtship displays incorporating colored ornamentations, suggesting the potential for the evolution of color vision across the family. With four pairs of eyes (anterior median – AME; anterior lateral – ALE; posterior median – PME; posterior lateral – PLE), how color vision evolves across these structures is still unknown. Previous work in *Hasarius adansoni* (tribe Hasariini) identified four visual opsins – the green-sensitive Rh1 and UV-sensitive Rh3 visual pigments expressed in the AMEs, with the remaining blue-sensitive Rh2 and UV-sensitive Rh4 visual pigments expressed primarily in the PMEs. To test whether this pattern of eye-specific opsin localization is common, and to identify species with opsin duplications that may suggest the evolution of additional color channels, we generated transcriptome datasets from 25 species of salticids representing ten tribes within the family. Based on this taxonomic sampling, the salticid visual system ground plan consists of three expressed opsins (Rh1, Rh2, and Rh3). There was evidence of gene duplication, and therefore potentially expanded spectral sensitivities, in species from five tribes (Aelurillini, Hasariini, Plexippini, Sitticini, and Spartaeini). Antibody-labeling studies suggest variation in opsin protein localization patterns across species, particularly for Rh1 and Rh3 in the ALEs and PLEs. The the AMEs from four species (representing tribes Baviini, Hasariini, Chrysillini, and Plexippini), all have similar Rh1 and Rh3 protein localization patterns to *H. adansoni*; in the species *Menemerus bivitattus* (tribe Chrysilinii) Rh3 was found in all of the eyes but with different expression patterns, while Rh1 was located in the AME, ALE, and PLE retinas.

C11 The Gluopsins: opsins without the retinal binding lysine

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Opsins allow us to see. They are G-protein-coupled receptors and bind as ligand retinal, which is bound covalently to a lysine in the 7th transmembrane domain. This makes opsins light sensitive. The lysine is so conserved that phylogenetic opsin reconstructions discard any sequence without that lysine. However, recently, opsins were found that function not only as photoreceptors but also as chemoreceptors. For chemo-reception, the lysine is not needed. Therefore, we wondered: Do opsins exists that have lost this lysine during evolution? To find such opsins, we built an automatic pipeline for reconstructing a large-scale opsin phylogeny. The pipeline can be easily used for other proteins as well. It includes collecting as many sequences as possible, aligning them, reconstructing the phylogeny, pruning rogue sequences, and visualizing the resulting tree. Our final opsin phylogeny is the largest to date with 4956 opsins. Among them is a clade of 33 opsins that have the lysine replaced by glutamic acid. Thus, we call them gluopsins. The gluopsins are mainly dragonfly and butterfly opsins. They are closely related to the RGR-opsins, and the retinochromes. Like those, they have a derived NPxxY motif. However, what their particular function is, remains to be seen.

C12 Cellular Scaling Rules for Amphibian Brains

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Relative brain size was traditonally used as the go-to proxy for cognitive abilities in comparative studies. In recent years, the focus has shifted towards better estimates of brain processing capacity, which is determined largerly by the number of neurons, inter-neuronal distances and the number of synapses. With the advent of the isotropic fractionator, it has become possible to reliably quantify numbers of neurons in whole brains with high throughput. Using this method, we analyzed the numbers of neurons and other cells in six major brain parts of 74 amphibian species representing all three amphibian orders - frogs (Anura), newts and salamanders (Caudata) and caecilians (Gymnophiona). The brain sizes varied starkly among the analyzed species, ranging from 8 mg to 312 mg. Number of brain neurons varied from 493,000 to 10,466,000. It was observed that newts and salamanders have significantly lower neuronal densities than frogs. These neuron numbers and densities will be compared with those of other vertebrates.

AUDITORY SYSTEM AND ACOUSTIC SIGNALING II

D18 The effects of multi-modal noise on conspecific call perception in the field cricket, *Teleogryllus commodus*

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For many species, the ability to detect behaviorally salient signals is essential for survival and reproduction. In field crickets, this involves the ability to perceive conspecific mating calls and predatory cues in complex acoustic conditions. Most studies on noise focus on energetic masking in which the noise stimuli overlaps in frequency with the signal of interest; however, informational masking in which noise does not overlap, affects an individual's ability to respond accordingly to a signal of interest has received very little attention in non-human animals. Noise can occur across a wide range of frequencies and can propagate through substrates. Insect sensory systems are morphologically and functionally diverse, capable of detecting both airborne and substrate-borne vibrations. By investigating the impact of unimodal noise on signals of a single modality, we overlook the complexity of how different noise sources may affect the ability of animals to recognize and localize salient sound sources. In behavioral experiments, we examined the ability of crickets to detect and localize cricket calling songs in the presence of different noise types. Our results show that localizing calls is negatively impacted by all noise types. We examined the ability of females to detect a target signal masked by different types of noise in extracellular neurophysiology experiments. Using signal detection theory, we found that neural detection thresholds were higher when noise frequencies overlapped with the conspecific calls. However, neural detection thresholds for target signals did not change in the presence of vibratory or ultrasonic noise. These results suggest that energetic and informational masking can have profound effects on the reproductive success of *T. commodus*.

D19 **Properties and variability in social acoustic communication of bottlenose dolphins**

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Bottlenose dolphins (Tursiops truncatus) have a complex vocalization system based on different types of sounds: whistles, clicks and burst pulses. Within dolphin pods, each individual has a specific whistle called signature whistle. The signature whistles are the dolphins' "names" which can be used to emit its identity or call a specific individual, playing an essential role in social interactions. These whistles may be differentiated by their distinctive frequency contour. Here, we study a pod of 5 dolphins living at the dolphin reef (Israel). A natural environment where dolphins are free to swim in the open sea at their own will. Thus, the place has the advantage of combining wild and captive-like conditions. Taking advantage of this unique environment, we use hydrophones and underwater cameras to record their vocalizations. We analyzed large datasets using methods of data mining, machine learning and dynamic time warping in order to extract and categorize the emitted sounds. Using this approach, we found that signature whistles have a stable frequency contour but can be modulated, most possible encoding additional information beyond the emitter's identity. In a second step, we studied the organization of their social acoustic interactions. We have found recurrent patterns in the dolphins' communication that could be the consequences of a set of rules that defines their social interactions (pragmatics). This approach is a first step in order to decipher the complex structure of the dolphin acoustic communication. In addition, correlating these data with the context will pave the way to uncovering further aspects of the pragmatics of their communication.

D20 **Study of bottlenose dolphin (*Tursiops truncatus*) acoustic communication during human-dolphin interaction using AI methods**

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Bottlenose dolphins (*Tursiops truncatus*) are known for their highly social complexity and associated cognitive abilities such as communication. The animal's acoustic repertoire is manifold, spanning from echolocation clicks for navigation to burst pulsed sounds and -signature and non-signature- whistles for social interaction. As gregarious animals, voluntary interactions with other species such as humans is not innocuous and vocal interaction may often result. However, this complex exchange remains understudied due to the strenuous experimental conditions that are faced when studying marine mammals in the wild. Our project aims at overcoming this challenge. We investigate dolphin acoustic communication in a semi-captive environment and with the growing use of novel computational methods such as automated clustering. At Eilat reef in Israel, an habituated group of cetaceans freely comes and leaves the pod while spontaneously interacting with the local care-takers. Daily activity is recorded with underwater hydrophones and video cameras, therefore providing us with unprecedented data. To elicit human-dolphin interaction, specific bells are rang at random times during the day to which dolphins respond, or not, by approaching and interacting with the "ringers". Recurrence of dolphin sounds are then clustered, classified and assessed with AI. Preliminary results have found that dolphins sometimes display vocal mimicry during inter-species interaction. Hence, we formulate the double hypothesis that these sounds produced by dolphins are non-random and that this repertoire may vary when interacting with humans. We hope with this unique approach that this study will contribute and disclose queries in the field of inter-species social interaction.

D21 The cortico-collicular axis and its role in sensory processing during vocalization

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In addition, to their ascending pathways that originate at the receptor cells, sensory systems are characterized by extensive descending projections. In the central auditory system, one projection, the so called cortico-collicular projection, which connects the auditory cortex (AC) with the inferior colliculus (IC), has received much attention due to its large size and complexity. This massive and diverse descending projection has been well-characterized both anatomically and functionally but not in the context of vocalization and active listening. Here we present the first characterization of colliculo-cortical activity during vocalization. For this purpose, we used parallel measurements of auditory cortex and midbrain signals in awake bats of the species *Carollia perspicillata*. Bats were positioned in the setup head-fixed and left to vocalize at their own volition. In addition, we studied the cortico-collicular responses to natural sounds presented at variable intervals with the aim of linking the phase of cortical oscillations to the strength of cortical and midbrain response. Our work sheds light into the role the mammalian cortico-collicular axis plays in sensory processing during vocal behavior.

D22 The effect of encoding sensory cue reliability on the function and development of the barn owl auditory system and sound localizing behavior

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In order to accurately perceive the world and respond accordingly, the brain must deal with noise inherent in sensory cues. One method is for the brain to learn which cues are reliable across contexts and rely on these cues in the future. To investigate this, we are investigating whether the reliability of binaural spatial cues drives the tuning of neurons that compute sound location in barn owls. Barn owls use the interaural time difference (ITD) to determine azimuthal location. Previous work showed that the signal-to-noise ratio of ITD varies across frequencies in a location dependent manner, based on the acoustical properties of the head. Thus, for a given location, certain frequencies convey ITD cues more reliably, and the neural tuning in the midbrain external nucleus of the inferior colliculus (ICx) reflects this pattern. We hypothesize that if the frequency tuning of the ICx is driven by cue reliability across locations, then altering the pattern of cue reliability should adjust frequency tuning. For this study, two barn owls were raised without the facial ruff, which modifies the pattern of ITD reliability. At adulthood, electrophysiological recordings of the ICx were performed. Results indicate that ICx neurons tuned to frontal locations are tuned to lower frequencies than previously reported, consistent with the change in ITD reliability induced by facial ruff cut. Additionally, recordings of the lateral shell of the core of the inferior colliculus (ICCls), immediately upstream of the ICx, show normal tuning to frequencies across the owl's hearing range. This suggests that the owls' midbrain still encodes ITD for higher frequencies, but are unused for sound localization. These data suggest that stimulus statistics are used to guide neural tuning.

D23 Temporal and social dynamics modulate the vocal repertoire of *Boana pulchellus*

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Animals communicate in diverse environments using complex sequences of acoustic signals. Mate choice is a key factor driving the evolution of these signals in many vertebrate and invertebrate systems. In anuran species for example, males produce advertisement calls to attract females, and female preference can lead to further evolutionary elaboration of these signals. Understanding the patterning of calling behavior provides insight into the evolution of acoustic communication across mating systems. Here, we examine how social feedback affects variability in temporal and spectral patterning of male calls in the South American treefrog, *Boana pulchellus*. We found that the complex calls of male *B. pulchellus* can contain three distinct note types that vary in frequency and duration, and that the calling sequences are dynamic and vary depending on the social feedback received. Taken together, our results suggest that there are temporal scales of consistency in calling behavior, and that these are modified by the context individuals are in.

D24 Visualization of grasp space and attention transitions in bats using echo reconstruction with acoustic simulation

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Until now, behavioral measurements such as obstacle avoidance behavior of bats have been studied mainly by measuring pulse and flight paths. On the other hand, in recent years, behaviors such as collisions against walls with smooth surfaces and misperception of objects of equal size but of different materials as objects of different sizes have been reported in echolocating bats. These findings suggest that the space that bats perceive through echoes is different from the space that we humans perceive primarily visually. Therefore, understanding the perceived space and information of bats based on echoes and interpreting their represented behaviors are necessary to elucidate the sensing strategies of bats. However, at present, there is no technology to measure faint echoes reaching bats in flight, and thus far, sensing strategies based on the echoes heard by bats and the information obtained from them have not been investigated. In this study, bat pulses and flight paths, which can be measured in behavioral experiments, were introduced into acoustic simulations to calculate echoes reaching bats in flight. As a result, we were able to visualize the space that bats grasp from their echoes ("echo space") and found that it differs from real space. We also found that bats smoothly move the object of their attention in flight prior to flight. By combining acoustic simulations with behavioral experiments in this study, we were able to reconstruct bat echoes and visualize the information that bats are reading from the echoes. In the future, it will be possible to use this method to model bat behavior decisions based on the relationship between the bat's input information (echoes) and its behavioral output (flight and pulses).

D25 Real-time whistle pitch-matching in wild nightingales

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Absolute pitch is the rare human ability to accurately identify the fundamental frequency of a given sound and precisely reproduce it. Pitch perception has been investigated in different animal species, but less is known about the real-time vocal imitation of a sound's pitch. The common nightingale (*Luscinia megarhynchos*) offers an ideal model to investigate auditory-vocal integration, as these songbirds naturally engage in song-matching duels against conspecifics, listening to and repeating the songs sung by their rivals. Additionally, these birds sing whistle songs consisting of frequency-unmodulated sounds that span across a broad distribution of fundamental frequencies (1000 – 900 Hz). To investigate whether nightingales are able to match the pitch of whistle songs, we recorded naturally interacting pairs of wild birds. We found that nightingales used these songs to match each other and that they targeted the fundamental frequency of their whistles to match the pitch of their rivals. To investigate to what extent wild nightingales are able to adjust pitch of whistle matches, we performed playback experiments exposing 13 wild birds to synthetic whistle songs of different pitches. We found that the birds' rate of whistle songs was increased by the synthetic whistle playbacks. Moreover, nightingales engaged with the stimuli by answering to and flexibly adjusting the pitch of their whistle to match the pitch of the playbacks. These results indicate that wild nightingales can spontaneously perform absolute pitch perception and production, flexibly controlling their vocal responses in real-time to precisely target auditory stimuli.

D26 Lack of *Fmr1*-gene impacts early development of vocal communication particularly in female mouse pups

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Fragile X syndrome (FXS) is the most prevalent form of inheritable cognitive disability, and also the most frequent monogenic cause of autism. Individuals with FXS are suffering from a range of symptoms including impaired cognition, communication, and social behavior. Similar deficits have been reported in the FXS mouse model (*Fmr1*-KO mouse). Alterations in vocal communication have been shown even in early postnatal development, however, mostly focusing on a narrow age range. In this study, mouse pup isolation calls from both male and female *Fmr1*-KO and sighted FVB (FVB.129P2-Pde6b^{+}Tyr$^{c\text{-}ch}$/AntJ; wt) control mice are examined in a developmental range from before hearing onset until shortly after (P2 – P12). We noticed alterations in the temporal domain of mouse pup isolation calls mainly in female *Fmr1*-KO pups. These alterations encompassed changes in the number of calls emitted and an on-average constant call duration. Most strikingly however, throughout their developmental trajectory female *Fmr1*-KO mice displayed an altered temporal organization of the calls they emitted, which was expressed by longer breaks between single calls. The in-depth analysis of the impact of the lack of the *Fmr1*-gene on the spectral call parameters is currently still ongoing. Our results will provide further insights into the alterations during early development of vocal communication in the *Fmr1*-KO mouse that might be beneficial for our understanding of the impaired communication in FXS.

D27 The role of motor cortex for the production of communication calls in the Egyptian fruit-bat

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Vocal communication is a widespread trait in the animal kingdom. Here, we studied the role of the orofacial motor cortex in vocal production in a social species of bats, the Egyptian fruit-bat. Using anatomical tracing, we identified a region of the motor cortex that sends direct, monosynaptic, projections to the motor neurons controlling the laryngeal muscles. Such a direct projection from the forebrain to vocal motor neurons is scarce in the animal kingdom. While it has been described in songbirds, among primates it has only been suggested to exist so far in humans. Electrophysiological recordings in that same region reveal increased neural activity in vocalizing but not in listening bats. Finally, we find that the activity of a subset of neurons is tuned to particular spectro-temporal modulations of vocalizations, suggesting a potential role in motor control. This study demonstrates the implication of orofacial motor cortex in the production of communication calls, in a mammal species for which a certain degree of vocal plasticity has been shown. These results open new avenues of research to further characterize the innate/learned and reflexive/volitional components of vocalizations and how it relates to the motor cortex activity and anatomical projections in that species.

D28 Echolocation reverses information flow in a cortical vocalization network

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The mammalian frontal and auditory cortices are important for vocal behaviour. Here, using local field potential recordings, we demonstrate that the timing and spatial pattern of oscillations in the fronto-auditory cortical network of vocalizing bats (*Carollia perspicillata*) predict the purpose of vocalization: echolocation or communication. Transfer entropy analyses revealed predominantly top-down (frontal-to-auditory cortex) information flow during spontaneous activity and pre-vocal periods. The dynamics of information flow depended on the behavioural role of the vocalization and on the timing relative to vocal onset. Remarkably, we observed the emergence of predominantly bottom-up (auditory-to-frontal cortex) information transfer patterns specific echolocation production, leading to self-directed acoustic feedback. Electrical stimulation of frontal areas selectively enhanced responses to echolocation sounds in auditory cortex. These results reveal unique changes in information flow across sensory and frontal cortices, potentially driven by the purpose of the vocalization in a highly vocal mammalian model.

D29 **Temporal coordination of *Danionella c.* vocalisations**

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*Danionella cerebrum (*Dc) is an emerging model organism for the neuroethology of acoustic communication. Dc possesses the smallest known vertebrate brain and is sufficiently transparent to allow for non-invasive optical interrogation of the brain even in adult animals. Additionally, male Dc are known to produce characteristic click bursts at 60 and 120 Hz. These bursts occur in various lengths, grouped into sequences, and often in the context of agonistic behaviour. Here, using a sub-wavelength acoustic triangulation setup and identity tracking in video recordings, we present the first characterisation of the vocal repertoire of Dc at the level of the individual. We describe the temporal coordination of vocal interaction, as well as its relationship to social hierarchy, and investigate possible behavioural motifs that accompany vocalisation episodes.

D30 **The neural basis of spectral prosody in avian vocal duets**

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It has been suggested that birds pay attention to the prosodic structure to coordinate their song during turn-taking. We hypothesize that prosodic cues, such as changes in the “intonation pattern” of song elements, facilitate duet coordination by predicting the boundaries of the partners‘ turns. Nuclei HVC plays a crucial role in arranging individual vocal emissions into sequences. We hypothesize that if prosodic cues are used by songbirds in vocal turn-taking, the production of song elements that differ in prosodic pattern should be controlled differently by HVC. To tackle these questions, we used duet singing White-browed Sparrow Weavers (*Plocepasser mahali*) as animal model. We recorded the individual vocal and HVC activity in both partners of duetting pairs and found that *P. mahali* duet songs are indeed organized into spectral prosodic patterns. Both male and female prosodic patterns were shown to alternate between each other. In addition, we found that in male *P. mahali*, HVC activity was different depending on the prosodic pattern of produced syllables. Median spike time relative to syllable onset was significantly higher in F-type syllables than in R-type syllables. This indicates, that the right HVC is active later during the production of F-type syllables than during the production of R-type syllables. While in R-type syllables, maximum frequency is rather low at the beginning of the syllable, in F-type syllables, maximum frequency is rather low at the end of the syllable. Given that low frequencies are produced by the right syrinx, lateralization of vocal motor control might explain our observations. We demonstrate the existence of spectral prosodic cues in birdsong and suggests song lateralization as the underlying neural mechanism for prosody generation.

D31 ***Danionella cerebrum* as a novel model system to investigate acoustic signaling and noise-coping mechanisms in vertebrates**

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A fundamental question for all acoustic communication systems concerns the biological function of vocal differentiation and how signal information is perceived and integrated by the nervous system. *Danionella cerebrum* (Cyprinidae) is a vocal and transparent miniature fish, which is a promising model to study the structure and function of the adult vertebrate brain and the vocal-auditory neural circuitry at the cellular level. This cyprinid species is genetically tractable (same family as zebrafish) and remains transparent throughout life, exhibiting the smallest known adult vertebrate brain. Our project relies on a set of behavioural, electrophysiological and imaging approaches to investigate the acoustic signalling system and the social function of sound production in this species. Moreover, the role of the acoustic environment shaping embryonic development, auditory processing and vocal behaviour is largely unknown in fishes. We also focus on the impact of environmental soundscape on embryonic development, establishment of auditory-vocal neural pathways, physiological stress and onset of vocal communication. We will present our ongoing research, preliminary findings, and discuss future research directions.

D32 **Stimulus-specific adaptation in the bat's frontal and auditory cortex**

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In humans, scream vocalizations have strong amplitude modulations (AM) at 30 to 150 Hz. These AM correspond to the acoustic correlate of perceptual roughness. In bats (species *Carollia perspicillata*), distress syllables also carry amplitude fluctuations at rates of approximately 1.7 kHz (> 10 times faster than in humans). The distress calls with these modulations are used more prominently by males and might signal a greater urgency, since they elicit larger heart rate increments than their demodulated versions. In order to study the neural processing of these two sounds (the distress calls with fast AM and the demodulated versions), we simultaneously recorded in two brain areas from the bat neocortex, the auditory cortex (AC) and the frontal auditory field (FAF), a frontal area responsive to sounds. We searched for stimulus-specific adaptation (SSA), which is described as the neuronal adaptation to a frequently presented stimulus (standard) yet responding strongly to an infrequent sound (deviant). The amplitude modulated natural calls and their demodulated forms were used as stimuli pairs. Our results show the existence of stimulus-specific adaptation in response to natural distress sounds produced by the bats. In addition, we describe that the dynamics of stimulus specific adaptation differs between frontal and auditory areas within the bat brain.

D33 **Brain-wide mapping of auditory-evoked responses in *Danionella***

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Our understanding of how auditory information is transformed along the auditory pathway is still limited and would greatly benefit from dense recordings of neuronal population activity across brain areas. Yet, the size and opacity of vertebrate brains make it technically challenging to monitor brain-wide activity at a temporally and spatially high resolution. To fill this gap, we recently introduced a novel model organism, the teleost fish Danionella cerebrum. Danionella combines small size, genetic tractability, and brain transparency with the complexity of a mature vertebrate and thereby enables new ways to explore distributed neuronal circuits. At the same time our data show that Dc exhibit complex behaviours including acoustic communication. Here, we developed a custom-tailored microscope (image transfer oblique plane microscope, ITOPM) to enable the recording of quasi-synchronous neural activity across the majority of the Danionella brain with single-cell resolution at 1 Hz volume rate during auditory stimulation. Using whole-brain oblique plane microscopy we identify Danionella's major auditory processing areas spanning hindbrain, midbrain and forebrain. Our data demonstrates that the acoustic processing of pure tones and pulses is already segregated at the level of the acoustic nuclei in the hindbrain. Finally, we show how tuning specificity and sensitivity of cells increases with the transition from hindbrain to midbrain and how reponses to vocalisation mimics systematically vary along the whole acoustic pathway.

D34 Auditory mechanics and morphometry of an insect's tracheal vesicles

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The tracheal vesicles of the field cricket dominate the proximity of the animal's sharply tuned auditory neurons. This sharp tuning is necessary for female phonotactic orientation towards the male's uncommonly pure-toned calling song, a behaviour essential to the commencement of reproduction. As such, sharp tuning to the carrier frequency of the male's calling song is integral to the second most precise directionality receiver known among invertebrates. Yet, after over four decades of enquiry, the biophysical cause of the neuronal stimulation remains enigmatic. Curiously, within this animal's unique auditory anatomy, the tonotopically arranged sensory neurons are separated from the sound inputs. Rather, they lie on the outside dorsal surface of a small chamber, the anterior tracheal vesicle, and the sound information enters an adjacent much larger cavity. The two vesicles are connected by two small apertures in their dividing wall. The proximal aperture is positioned at the level of the sensory neurons. We hypothesise these geometries contribute to the frequency tuning of the neurophysiology, and/or to signal transduction in the auditory pathway. Micro-CT imaging, segmentation, and 3D-reconstruction have revealed the morphometric measurements of these structures for the first time. Incorporating this data into a 3D model and applying numerical simulations under behaviourally relevant sound frequencies should indicate the auditory mechanics of the tracheal vesicles; again, for the first time. Preliminary results suggest a possible sharp tuning in velocity at the proximal aperture, and in velocity phase differences between vesicles, at the carrier frequency of the male's mating song. Behavioural significance to any conclusive results will be discussed.

D35 Acoustic communication in early cretaceous crickets

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The Crato formation of Brazil's Araripe basin has yielded exceptionally well-preserved fossils from the Early Cretaceous (Aptian), 112-102 million years ago. The limestone fossils include a plethora of insect remains. Here we report on cricket fossils with striking similarities to extant species, e.g. field crickets. This includes wing structure, exhibiting harp and mirror fields in males, characteristic of stridulation for acoustic communication. This suggests that acoustic communication was present in Early Cretaceous crickets and resembled extant species-specific acoustic communication. Despite excellent preservation, stridulatory file structure is not recognisable in the available specimens, but ridges of some file teeth appear discernible. Together with other morphological details, this suggests that sound frequency of the fossil cricket calling song was in a range similar to extant crickets. The following list compares data for a fossil specimen and corresponding average data for field crickets.

Fossil cricket: body length 18.7 mm, wing length 8.9 mm, stridulatory file length 2.3 mm, file tooth spacing 50 micrometers

Gryllus campestris: body length 21 mm, wing length 14 mm, stridulatory file length 4.4 mm, file tooth spacing 30-45 micrometers

While stridulation in orthopteran insects has been demonstrated for the Jurassic previously (Gu et al. 2012, PNAS 109, 3868), the highly modern features of Early Cretaceous crickets some 50 million years later are striking. This observation documents the existence of acoustic communication, and thus stridulation as well as hearing, well before the advent of bats around 52 million years ago. The presence of bats had previously been assumed to be a major reason for the evolution of insect hearing.

↑UP

D36 **Conserved vocal central pattern generator in genus *Xenopus***

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Courtship behavior of animals encodes information about the species' identity. Although the exact cellular and synaptic properties of the neural circuitry underlying the courtship behavior are likely to be species-specific, the general architecture of the courtship neural pathways may be conserved across closely related species. Here, we examined phylogenetic conservation of the central vocal pathways in five species of the genus *Xenopus*. Male Xenopus produce species-specific advertisement calls made of a series of clicks underwater to attract females of the same species. Although the acoustic morphology of the advertisement calls is diverse, the temporal organizations of the calls within the genus can be divided largely into three categories: fast trills containing clicks repeated at >60Hz, slow trills with clicks repeated at <30Hz, and biphasic calls that contain both fast and slow trills. Previously, we showed that the biphasic advertisement calls of male *Xenopus laevis* are generated by anatomically distinct fast and slow trill central pattern generators (CPGs) contained in the brainstem. In this study, we evoked fictive vocalizations from the isolated brains of two fast trillers (*X amieti, X. cliivi*), one slow triller (*X. tropicalis*), and one biphasic triller (*X. petersii*) and compared the functional and anatomical organizations of the vocal CPGs to those of male (biphasic triller), female (slow triller), and testosterone-treated female *X. laevis* (biphasic triller). The results showed that the location and the function of the fast and slow trill CPGs are conserved across species, and each species have one or both CPGs. The results suggest that the species-specific vocal neural circuitries are built upon conserved fast and slow trill CPGs.

D37 **Does male size really matter? – A study on correlations between calling song frequency and body size parameters within and across cricket populations**

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It was suggested that female field crickets prefer lower-tuned calling songs reflecting sexual selection by female choice for larger males as mating partners. However, several studies failed to show a correlation between male body size and the sound frequency of their calls. Here we analysed how different body size indicators (mass, length and width) correlate with the fundamental frequency of the calling song within and across two populations of the field cricket *Gryllus bimaculatus*, that differ significantly in size. One test group of relatively small animals came from commercial breeders and were bought as adults from pet shops (S) while another group with relatively large individuals came from a long-term inbreed stock of our lab colony (L). The data show correlations between all measured body size parameters within each group (S, L) and also across all animals of the two populations combined (S+L). However, the fundamental frequency of the calling song did not correlate with any of the body size parameters within either of the two populations itself (S, L). Only when the data of both populations were pooled together (S+L), a significant correlation between body size and call frequency was found. The fact that females usually can only select between males of their own population makes it very unlikely that they can use the calling song frequency as indicator for male body size. In summary, our study demonstrates that a correlation between two parameters at the species level does not necessarily mean that a similar correlation can be found in each subpopulation. Moreover, our data highlights the rather general analytical problems of sufficient parameter range and sample size as well as hidden subgroups within data sets for statistical correlation analysis.

ELECTROSENSORY SYSTEM II

E13 Complex frequency modulations in freely interacting electric fish, *Apteronotus leptorhynchus*, recorded in their natural habitat

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Behaviors used for communication should be sufficiently conspicuous to be received effectively. But it is the signals that are subtle and hard to classify that are most challenging to understand for us humans. *Apteronotus leptorhynchus* is a nocturnal weakly electric fish that uses its electric organ discharge (EOD) to locate objects and to communicate with conspecifics. Signals commonly associated with communication ('chirps' and 'rises') can be identified by their stereotyped EOD frequency (EODf) modulations during mating and competition. Longer and increasingly complex EODf modulations have been rarely observed and described, and their natural, evolutionary function is not well understood. Studies that tried to investigate these modulations took place in laboratory conditions with unnatural stimulus regimes, where observed behaviors may vary greatly from natural behaviors. For a natural population of *A. leptorhynchus*, recorded in a neotropical stream in Colombia using an array of 64 electrodes, we find a great diversity of EODf modulations, including complex EODf modulations lasting many minutes. Some of these signals occurred synchronously in two animals. Based on the spatial distribution of EOD amplitudes over the recording electrodes we estimated the position of the fish and analyzed their movements during these EODf interactions. In a laboratory experiment where a confined fish is stimulated with an artificial electric field simulating a conspecific, we were able to reproduce these correlated interactions in EODf, but only for stimuli that are close in frequency to the EODf of the target fish. Our analysis of field data together with dedicated laboratory experiments is a first step towards an understanding of these complex signals in their natural context.

E14 Magnocellular mesencephalic nucleus in *Apteronotus albifrons*

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Gymnotiform electric fishes possess independent tuberous electrosensory pathways for amplitude and timing information. In the timing pathway, timing of each electric organ discharge (self-feedback electrosensory signals) is encoded accurately in the timing of action potentials first in the electrosensory afferent fibers, second in the spherical cells in the medulla, and finally in the phase-locking neurons in the midbrain. These midbrain phase-locking neurons, which provides inputs to phase comparator neurons within the midbrain, constitute a layered structure in wave species and a hypertrophied non-layered structure in pulse species. In this study, a high-frequency wave species, *Apteronotus albifrons*, is found to possess a hypertrophied midbrain structure resembling the mesencephalic magnocellular nucleus that is typically found in pulse species. In this structure of *A. albifrons*, there are two types of phase-locking neurons of large size and phase comparator neurons of small size as in other gymnotiform fishes. The phase-locking neurons of *A. albifrons*, however, exhibited complete 1:1 firing even in individuals with electric organ discharge frequencies as high as 1300 Hz. Receptive field of the phase-locking neurons were found in the anterior body surfaces reflecting the distribution of tuberous electroreceptors on the body surface.

E15 Object size and distance discrimination strategies in *Gnathonemus petersii*

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In active sensing animals invest energy to perceive. Amongst these, weakly electric Mormyrid fish were found to gradually adapt this sensing strategy to aid this process. We here are interested in discovering how and when these fish (*Gnathonemus petersii*) adapt their electromotor strategy to gain sensory information when the task requires the detection and comparison of multiple stimulus features. We present here the data of five fish that were trained to swim through a corridor and turn to the correspondent side of the target object. The task consisted in discriminating an object by its size. For this, we trained the fish to solve the task using two congruent cues: relative distance and size. Three of these fish successfully completed the training session. Later in a test phase, for those three fish, the distance cue was inverted to assess whether they had learned the relative distance or the size cue. Fish discriminated the object size either when the relative distance of the two objects was the same or when they differed by no more than one centimetre. Nevertheless, for the majority of the test trials, fish seemed to solve the task based on relative distance; which might be due to the characteristics of the training experience. Additionally, to unravel the fish's motor strategy among different conditions, we generated heatmaps of the head location and quantified the stereotyped motor patterns throughout the trials and along the arena to address if different motor strategies are recruited between learning strategies and discrimination context**.**

E16 Is melatonin enough? Central and peripheral actions of melatonin on the electric behavior of *Brachyhypopomus gauderio*

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Living organisms display molecular, physiological and behavioral endogenous rhythms which are synchronized with environmental cycles and social stimuli. Biological rhythms confer an evolutionary advantage allowing a more efficient energy allocation for environmental changes. In vertebrates, rise in melatonin concentration coincides with the onset of the night and persists throughout its duration. Melatonin is mainly synthesized in the pineal gland, the main source of circulating hormone, with other sites of production in the brain, associated with paracrine or even synaptic melatonin actions. *Brachyhypopomus gauderio* is a South American pulse type weakly electric fish characterized by the constant emission of electric pulses with a stable basal frequency, that result from the activation of an electrogenic pathway. A medullary nucleus, the "pacemaker nucleus" provides a one to one control on EOD emission. EOD basal rate (EOD-Br) is modulated by environmental and social information and part of a behavioral trait known as electric behavior. A daily rhythm in EOD-Br has been reported for this species, consisting on a nocturnal increase () that depends on the activation of the 1B melatonin receptor. The EOD-BrNi persists in constant light/darkness conditions, provided social context is maintained. In nature EOD-Br daily cycle occurs in natural constant darkness conditions and is strongly modulated by social context. A daily cycle in EOD-Br variability has also been described in a related species. The role of melatonin in the modulation of electric behavior was analyzed by daytime IP administration to isolated individuals as well as through in vitro experiments on brain stem slices, showing its key role for the nocturnal physiology of the species.

E17 Optimal electrosensing in mormyrid electric fish

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Mormyrid electric fish generate pulsed electric fields, and they identify the resistive and capacitive properties of nearby objects by detecting small microvolt and microsecond distortions in these fields. They do this despite much larger external modulations caused by self-motion, boundary effects and changing water conductivity. Mormyromast electrosensors on the skin of the fish are highly sensitive to the minute field distortions produced by objects. Although it has been suggested that particular features associated with the peaks of the electric pulse waveform are critical for object detection, the precise features used have not previously been characterized. By combining experimental measurements, neural network modeling and an electric field model, we identified temporal filters that characterize the operation of mormyromast electrosensors and developed a novel adaptive model of their sensory processing. Our electric field model unifies previous work into a fast and scalable analytic framework that allows us to generate large simulated data sets for developing and analyzing neural network models. We characterized the mormyromasts as temporal convolutional filters optimized to extract behaviorally relevant stimuli. We also fit convolutional filters to experimentally measured responses of the mormyromasts and found results that agree well with those obtained by optimization. Our mormyromast model continually adapts to slowly varying input modulations, in line with experimental findings, and it provides a basis for modeling downstream computation in the cerebellar-like circuits of the fish's electrosensory lobe.

E18 A spark in the dark - activity rhythms of African weakly electric fish

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Animal life rhythms have been researched most thoroughly in a small group of model animals and a systematic understanding of behavioral rhythms remains lacking for many species. This knowledge gap includes many aquatic organisms whose natural behaviors and environmental conditions are much less well known than those of terrestrial animals. Weakly electric fish are a good example of animals that elude traditional sampling techniques as they are generally considered nocturnal and prefer structurally complex habitats. However, their electric organ discharges (EODs) can easily be detected non-invasively in a water body and reveal their behavioral patterns I explored patterns of activity and habitat use in two species of mormyrid weakly electric fishes under controlled laboratory conditions and in a swamp lagoon in Uganda, using self-built recording devices. In the laboratory, fish remained largely inactive and sheltered during the light phase, and increased their EOD rates and exploration behavior drastically with the onset of the dark phase. In the wild, fish dwelled mostly under floating vegetation during the day, where they showed higher levels of diurnal activity than in the laboratory, and ventured out into open areas of the lagoon at night. Interestingly, the active phase of the fishes coincided with severely low dissolved oxygen concentration in their natural habitat, and the nocturnal increase of activity was less pronounced in the wild than in the laboratory. The comparison of laboratory and field data suggests that light plays an important role in the regulation of activity in these fishes, and that natural activity patterns are more varied than those observed in the laboratory.

E19 A bespoke and affordable methodology for characterising the electrical environment

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For over half a century, researchers have known that a number of aquatic species possess an electric sense. So far, most studies have focussed on the physiology and neuroethology of electroreceptors in vertebrates, such as the ampullae of Lorenzini of sharks and rays. Thus, there stands a breadth of knowledge detailing the behaviours and mechanisms employed by electrosensitive organisms. At the physical ecological level however, there is limited information about the electric environment underpinning electroreception. To rectify this in the freshwater environment, we are developing a methodology for measuring biogenic and abiotic sources of electric fields, using an inexpensive, portable, and easily reproducible set-up consisting of commercially available hardware and custom Python software to record and characterise electrical signals. Here, we present the methodology as well as example recordings of freshwater organisms. Using this methodology, we aim to compile a library of electric recordings to provide contextually relevant stimuli for behavioural experiments but also, importantly, to map the aquatic electrical environment. This approach aims to deliver a more comprehensive context to research into electroreception. We hope that these data will help bridge the gap between mechanistic and ecological research in the burgeoning field of electric ecology.

E20 Extremely high numbers of brain neurons in weakly electric fish

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The ability to sense weak electric fields is widespread and likely ancestral in fish. This capacity has been lost in teleost fishes and afterwards independently reacquired in several teleost lineages. Some of them evolved passive electroreception, while others can produce weak electric fields and use them to orient in their environment and communicate with conspecifics. Here we used the isotropic fractionator to compare the numbers of neurons and their distributions across major brain parts in basal ray-finned fishes, teleosts without the ability to sense electric fields and those that secondarily evolved passive and active electroreception. We show that passively electroreceptive basal ray-finned fishes have rather modest numbers of brain neurons and there are no consistent differences between the brains of teleosts with and without passive electroreception. An enlarged cerebellum coupled with increased neuron density has evolved at least two times independently in weakly electric teleost fishes. In extreme cases, their cerebellum and telencephalon contain more than 95% and less than 0.5 % of brain neurons, respectively. Mormyrids are highly encephalized and feature the highest known cerebellar neuron densities, resulting in brain neuron counts equal to or greater than some large mammals (including monkeys) with much bigger brains. These findings strongly suggest that active electroreception is very computationally demanding.

E21 **Neuronal noise and heterogeneity increase the dynamic range for encoding electrosensory stimuli**

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P-unit electroreceptors of the weakly electric fish *A. leptorhynchus* encode a broad range of sensory stimuli in the context of electrolocation and electrocommunication. The behaviorally relevant stimulus amplitudes range over about four orders of magnitudes. While P-unit encoding has been extensively studied for relatively strong stimuli, little is known about the encoding of behaviorally relevant, weak stimuli. Here we analyze the encoding of electrosensory stimuli with a wide range of stimulus amplitudes both in electrophysiological recordings and in simulations of realistic leaky integrate-and-fire models. Our results demonstrate that weak stimuli can be encoded by large homogeneous P-unit populations. We further find that noise by means of suprathreshold stochastic resonance (SSR) is crucial in the P-unit population for improving information transmission. A combination of stimulus amplitude, noise strength, and population size shapes SSR and defines which stimulus amplitude is encoded best by a P-unit population. These interactions can be explained by the balance between two opposing noise-related mechanisms in sensory encoding. On one hand, increasing noise decreases signal-to-noise ratio and thus transinformation. On the other hand, noise decreases the redundancy in the population, improving signal transmission. We also show that higher levels of intrinsic noise in a heterogeneous population increases the dynamic range towards higher stimulus amplitudes in large populations by trading off coding accuracy for weaker stimuli. Our results show how noise, heterogeneity and population size are optimally adapted to allow for encoding a wide range of behaviorally relevant electrosensory stimuli.

E22 **Descending pathways promote neural response heterogeneities to behaviorally relevant stimuli**

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How the brain processes incoming sensory stimuli to gives rise to behavior remains poorly understood in general. Here we performed multi-unit recordings from sensory pyramidal cell populations within the electrosensory lateral line lobe (ELL) of awake behaving weakly electric fish (*Apteronotus leptorhynchus*) in response to stimuli whose amplitude (e.g., envelope) varied. Previous studies have shown that envelope stimuli carry critical information as to the distance between two conspecifics and give rise to well-characterized behavioral responses. Our results show that pyramidal cell responses to envelope stimuli were highly heterogeneous. Moreover, pharmacological inactivation of descending pathways from higher brain areas (i.e., feedback) rendered the neural population more homogeneous such that most neurons responded in phase with

the stimulus but also introduced redundancy. As such, an optimized decoding scheme that considers response heterogeneities is necessary to optimally encode envelope stimuli by a heterogeneous neural population in order to generate behavioral responses to these. Moreover, using an evolution algorithm revealed that a combinatorial code could explain the animal's behavioral responses. Our results therefore not only reveal a novel function for feedback pathways but also provide critical insight as to how behavioral responses to envelope stimuli are generated.

E23 Receptive field sizes and neuronal encoding bandwidth are constrained by axonal conduction delays

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Convergence is a fundamental motif in neuronal networks. A postsynaptic neuron integrates the output signals of a number of presynaptic cells. The general assumption is that convergence leads to a better representation of the stimulus since, assuming independent noise, the noise in the integrated signal will be scaled with the square root of the population size. While of course true, it implicitly assumes that the input from the presynaptic neurons reaches the integrating neuron synchronously. In real neuronal networks, however, the traveling of action potentials down an axon takes time and thus information will not reach the postsynaptic neuron synchronously. The spikes of one end of the receptive field may lead while those from the other end may lag behind. Effects of such spread in arrival times on neural coding has, to our knowledge, not been analyzed so far. Here we investigate the interplay of receptive field size, conduction delays and stimulus dynamics in the electrosensory system of the weakly electric fish *Apteronotus leptorhynchus*. Generally, increasing the population size increases the amount of information about the stimulus that is contained in the population response. At the same time, increasing the spread of conduction delays, as unavoidably happens by increased receptive field sizes, severely affects the representation of high-frequency stimulus components and thus limits the maximum extent of receptive field sizes. Our results from experimental data and model simulations show that stimulus dynamics, receptive field sizes, and conduction velocity constrain the design of nervous systems.

E24 Serotonergic modulation of population coding

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Organisms have developed coding strategies that are optimized to best encode highly dynamic natural stimuli in order to survive. This process is thought to be mediated by neuromodulators such as serotonin. In the weakly electric fish Apteronotus leptorhynchus, serotonin has been shown to optimize neural and behavioral responses to stimuli associated to a specific social context. However, these results were based on single neurons recordings. As such, whether and if so, how serotonin optimizes coding at the population level remains unknown. Here, we investigated how serotonin modulates population coding of social stimuli using simultaneous electrophysiological recordings. Our results show that release of serotonin selectively enhances responses of a subset of neurons and increases their correlated activity. Further inspection of correlated activity shows that serotonin increases signal correlations, causing neural responses to be more homogenous during stimulation. While redundancy between neural responses increased, our results also show that a decoder that is based on coincidence detection performed better after serotonin release, which agrees with the animal's behavioral response. Our results provide, for the first time, experimental evidence that the role of serotonin is to enhance neural responses to behaviorally relevant stimuli by increasing synchrony in neural populations.

SOCIAL BEHAVIOR AND NEUROMODULATION II

F19 Neurogenomic response to aggression in females of the Siamese fighting fish

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In many species, both males and females engage in agonistic encounters. However, the proximate mechanisms (i.e. genetic, hormonal and neuronal) mediating the display of aggression have been more extensively studied in males than females, with divergent results presented in the literature. In the Siamese fighting fish *Betta splendens*, males were selected for winning paired-staged contests for more than six centuries, originating short-fin varieties known as "Plakat Morh" (fighter strain). Interestingly, females of the fighter strain, which were not the target of this selection, are also more aggressive than wild-type females. In this work we compare the behavioural and neurogenomic response of females to the mirror test in both wild-type and fighter strains. Whole-brain gene expression analyses revealed that the selection for aggression induced a markedly different neurogenomic baseline state in fighter females when compared with wild-type conspecifics. However, within strains, the response to the aggression test showed a low number of genes being differentially expressed. These results taken together with previous work in males show that fighter and wild-type strains differ in their neurogenomic baseline in both sexes, and only one gene, *npas4*, was found up-regulated in both fighter males and females. This study presents the first transcriptomic comparison of females for *B. splendens* strains in response to an aggression test, a promising species for the investigation of the mechanisms of aggression in vertebrates.

F20 Probing the neurobiological basis of sex differences in visually-evoked aggression in the Siamese fighting fish *Betta splendens*

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Visual information plays a prominent role in driving human social behaviors, yet in commonly used rodent and fly models, such behaviors are evoked largely by chemosensory cues. How visual cues influence social behavior is therefore relatively unexplored. My work uses Siamese fighting fish (*Betta splendens*), which have been bred for hundreds of generations for robust male aggression, to understand how visual cues drive aggressive behavior. Because Betta aggression is largely restricted to males, I am able to explore how the visual cues that drive aggression are transformed in males and females to produce distinct behavioral responses. I have developed behavioral assays that reliably produce aggressive display behavior in Betta, which has allowed me to identify large quantitative sex differences in the behavior. Using molecular activity marker pS6, I have identified several brain regions exhibiting differential activity in males in females responding to a male conspecific. My tracing experiments with cholera toxin b subunit (CTb) have delineated putative circuits linking these regions to known visual areas.

F21 Dynamics of the steroid response to an aggressive challenge in a wild-type and fighter-selected strain of *Betta splendens*

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Engaging in aggressive interactions increases peripheral androgen secretion in many species. However, the functional role of this increase, in particular in the ongoing contest or as mediator of behavioural changes in future interactions, remains controversial. Here, we studied the temporal variation in plasma and water-borne levels of KT in response to a mirror-challenge in a wild-type and a fighter-selected strain of the Siamese fighting fish *Betta splendens*. Possible differences in fight performance between males of fighter and wild-type relate to variation in the temporal dynamics of the androgen. To test this hypothesis, male fighter and wild-type were exposed to their mirror image and allowed to fight for variable periods of time. Blood levels of KT were quantified between 2 to 60 min fights. To study whether if the KT response could be detected in the fish holding water, levels of the hormone were quantified between 10 to 360 min. A significant increase in plasma KT levels at all time intervals was detected, as compared to controls, and as early as 2min after the beginning of the fight. KT levels in water were generally elevated in the experimental groups but there was no positive correlation between plasma and water-borne KT levels. However, average levels of KT and the temporal pattern of response both in water and in plasma was similar in fighter and wild-type males. The results suggest that 1) selection for winners did not impact the temporal pattern or average levels of the KT response to aggression; 2) water-borne KT levels are appropriate to test differences between groups but do not reliably correlate with plasma levels. Further, the fast androgen response in plasma suggests a novel non-endocrine mechanism of activation of the HPG axis.

F22 To flex or flee: Investigating defense behavior and its neural control during symbiotic interactions in rove beetles

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How do organisms evolve to interact with one another? What changes to neural structure and function enable adaptive interspecies interactions? We are using rove beetles as a model system to investigate the behavioral motifs and neural architecture underlying the evolutionary origins of symbiotic behavior. Free-living beetle species have repeatedly evolved into symbiotic myrmecophiles that socially interact with specific host ant taxa. To assess the origins of such specialized behavior, we have quantified the behavioral motifs underlying beetle-ant interactions in the ancestral, free-living species, *Dalotia coriaria*. *Dalotia* possess a flexible abdomen with a benzoquinone-secreting chemical defense gland that functions as a potent ant deterrent. Using multi-view behavioral imaging and tracking, we find that *Dalotia* exhibit a robust abdomen flexion defense response such that flexion angle increases as the distance between *Dalotia* and ant decreases. Such behavior is olfactory mediated, as anosmic beetles (orco mutants) have a significantly reduced flexion angle upon ant approach. We are also examining changes in neural architecture of the central nervous system that may mediate the transition to sociality. We have developed a comprehensive map of major brain regions and the ventral nerve cord (VNC) of both the free-living, *Dalotia*, and the symbiotic myrmecophile, *Sceptobious lativentris*, species. Our preliminary data demonstrate species-specific differences in the pattern of neuromere (segmented modular ganglia) fusion in the VNC, potentially underlying differences in the innervation pattern of the VNC-gland module. Our behavioral and anatomical work begins to lay the foundation for using rove beetles to examine the neural basis for evolution of interspecies interactions.

F23 Neural correlates of natural social behavior in freely-moving macaques

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Success in group-living primates, including humans, requires navigating complex and diverse social relationships. Current understanding of the neural mechanisms underlying primate social behavior derives almost exclusively from highly-constrained and scripted laboratory tasks. Precisely how the primate brain dynamically navigates species-typical social interactions, in all their richness and complexity, remains largely unknown. Here we leverage new technology to investigate the neural correlates of an unprecedentedly diverse array of species-typical behaviors in freely-moving, socially-interacting rhesus macaques. We recorded single neurons from the mono-synaptically connected inferior temporal area TEO and prefrontal area 45 in two male macaques (n=6,129 units, 20 sessions) while they interacted with their female partner, and varied the identity of neighboring monkeys. Monkeys exhibited a rich behavioral repertoire of up to 29 different behaviors also observed in the wild, including grooming, foraging, aggression, and mating. The behaviors of the recorded monkeys were reliably decoded on a second-by-second basis from the simultaneous activity of a few hundred neurons (>70% accuracy, chance ~6%) –even during behaviors extending over many minutes where the sensorimotor environment changed considerably. Remarkably, single neurons widely encoded both the subject's behavior (>80% selective units) and the social context in which it occurred; e.g.: whether grooming was reciprocated, initiated, or whether it occurred after a threatening event, and the identity of neighboring monkeys. Moreover, neural activity was also modulated by the actions of the female partner, providing parallel representations of the behavioral states of both self and other. Surprisingly, decoding performance and single unit responses were similar in temporal and prefrontal cortices, with important implications for theories of social information processing. These preliminary results demonstrate that neural ensembles in the prefrontal and inferior temporal cortices of macaques carry information about species-typical social stimuli, behavior, and contexts required for success in the wild.

F24 Pre-copulatory reproductive behaviours are preserved in *Drosophila melanogaster* infected with bacteria

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The activation of the immune system upon infection exerts a huge energetic demand on an individual, likely decreasing available resources for other vital processes, like reproduction. The factors that determine the trade-off between defensive and reproductive traits remain poorly understood. Here, we exploit the experimental tractability of the fruit fly Drosophila melanogaster to systematically assess the impact of immune system activation on pre-copulatory reproductive behaviour. Contrary to expectations, we found that male flies undergoing an immune activation continue to display high levels of courtship and mating success. Similarly, immune-challenged female flies remain highly sexually receptive. By combining behavioural paradigms, a diverse panel of pathogens and genetic strategies to induce the fly immune system, we show that pre-copulatory reproductive behaviours are preserved in infected flies, despite the significant metabolic cost of infection.

F25 Ethogram of mouse sexual behavior

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Mouse sexual behavior has a complex structure that involves stereotypical actions and concrete neural activations and dynamics. The strong interplay between neuronal states and actions –combined with its ecological relevance– makes it an ideal model behavior to better understand how such complex tasks are organized and generated. However, due to the difficulty of quantifying unrestrained social interactions, the need for large behavioral data sets, and the lack of a unified set of behavioral motifs during copulation, mouse sexual behavior has been largely understudied and uncharacterized. Therefore, building an ethogram of mouse sexual behavior is an important step forward in understanding the behavior and how it arises. Using a large longitudinal dataset collected by our lab and behavioral quantification based on accelerometer, video and manually annotated data, we are currently building an ethogram of mouse sexual behavior. In this work, we will present preliminary results on the stereotypical and experience dependent characteristics of mouse sexual behavior, as well as a Markov model for the transitions that occur between the different motifs. We believe our efforts may have multiple implications, starting with a deeper understanding of the behavior, to our ultimate goal of linking behavior to neural dynamics.

F26 Anatomical and electrophysiological characterization of hypothalamic neurons involved in female sexual behavior

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Throughout the reproductive cycle, fluctuating levels of sex hormones coordinate female behavior with their reproductive capacity by modulating the activity of neuronal circuits expressing their specific receptors. The ventrolateral division of the ventromedial hypothalamus (VMHvl) is rich in neurons expressing receptors for sex hormones and its function is intimately linked to female sexual receptivity. However, recent findings suggest that the VMHvl is functionally heterogeneous. To gain insight into the underlying underpinnings of such functional heterogeneity, we used whole electrophysiological recordings, viral tracing, and neuronal reconstructions tools to characterize the electrophysiological and anatomical properties of individual VMHvl neurons in naturally cycling females. We found that the properties of progesterone receptor expressing (PR+) neurons, but not PR– neurons, depende systematically on the neuron's location along the anterior-posterior (AP) axis of the VMHvl and the phase within the reproductive cycle. In addition, anterior and posterior VMHvl PR+ neurons exhibit different connectivity patterns. The observations reveal the existence of phenotypes within the PR+ subpopulation, supporting the anatomic and functional subdivision of the VMHvl and its possible role in the orchestration of different aspects of female socio-sexual behavior.

F27 Should I mate or should I reject? A novel role for the anterior VMHvl in the cyclical control of female rejection behavior

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In females of most species, cyclic fluctuations in the levels of sex hormones closely coordinate sexual receptivity with reproductive capacity. This is clearly demonstrated in rodents, as only female mice in the fertile phase respond to male copulation attempts with lordosis, the sexually receptive posture, and have sex. Outside the fertile period, copulation never occurs because females do not perform lordosis and reject male copulation attempts by running away, adopting defensive postures or even punching and kicking the male. Because these behaviors are executed in response to the threatening actions of others, rejection behaviors can be characterized as defensive. Sadly, while a lot of attention has been dedicated to the study of female receptivity/lordosis, rejection/defensive behavior has been largely ignored and often regarded as a mere lack of receptivity. The role of the ventrolateral region of the ventromedial hypothalamus, in particular its progesterone receptor expressing population (VMHvl.PR+), in the cyclical control of lordosis is well established. Due to recent studies showing its involvement in other facets of female behavior, we wondered if this hypothalamic nucleus could also be involved in rejection behavior. Here we propose a new role for the anterior VMHvl (aVMHvl) in the cyclical control of female rejection behavior. Evidence for this hypothesis initially came from the observation that the aVMHvl is more active when non-fertile females interact with a male, an interaction dominated by rejection behavior. Second, using calcium imaging methods to monitor the activity of the VMHvl.PR+ population across the reproductive cycle, we found spatial segregation in the response profile of these neurons: notably, lordosis-related activity seems to be localized to PR+ cells in the posterior region of the VMHvl; in contrast, aVMHvl.PR+ neurons are active when non-fertile females reject male copulation attempts. Importantly, in fertile and receptive females, aVMHvl.PR+ neurons are silent in response to male copulation attempts. Electrophysiological characterization of the properties of PR+ neurons across the reproductive cycle revealed periodic, spatially segregated changes in the incoming synaptic input. In particular, aVMHvl.PR+ neurons seem to experience profound changes in the amount of inhibition they receive, such that the excitation/inhibition (E/I) ratio of these cells in non-fertile females is much higher when compared to fertile females. This change in E/I balance suggests that aVMHvl.PR+ neurons might be more active in non-fertile females, corroborating the calcium-dependent recordings. To causally relate these findings, we performed localized optogenetic stimulation of aVMHvl.PR+ neurons in fertile females, which normally exhibit very low levels of rejection. As expected, our manipulation led to increased rejection behaviours in fertile females and to a reduction in the percentage of females that mated with the male. These results lead us to propose a dual, spatially segregated and sex-hormone-dependent role of the VMHvl.PR+ population in the bidirectional control of female sexual behavior. When non-fertile, sex never occurs due to the combined incapacity of the pVMHvl.PR+ population to facilitate lordosis and the rejection behavior induced by aVMHvl.PR+ neurons. In contrast, when fertile, the aVMHvl.PR+ is mostly silent, such that attempts of copulation are not rejected and lordosis can be expressed via the pVMHvl.PR+ population. Overall, our study shows that the VMHvl may have distinct subdivisions that are relevant in the context of female rejection and mating behaviours and uncovers the role of a novel subpopulation of aVMHvl.PR+ neurons.

F28 Neural mechanisms of juvenile aggression

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Aggression in juveniles is an evolutionarily adaptive behavior documented in many species, but the neural mechanisms behind these displays are poorly understood. Mimetic poison frog (*Ranitomeya imitator*) tadpoles live in small pools with limited resources and have evolved offensive aggressive tendencies towards conspecific tadpoles. Tadpoles will attack, kill, and cannibalize other tadpoles as a primary resource defense mechanism. I examined the neural basis of neonate aggression in these tadpoles by comparing individuals that were placed in aggressive encounters and individuals placed in an environment with a non-threatening stimulus. I first did a longitudinal study to determine that the tadpoles were most aggressive when they were around five or six weeks old. I then compared patterns of generalized neural activity using immunohistochemical detection of phosphorylated ribosomes and a candidate neuropeptide, arginine vasotocin. Vasotocin cells were not seen to be more active in aggressive tadpoles. I then examined neural activation across several brain regions suspected to be involved in aggression, and found that the anterior amygdala had the most neural activation in aggressive tadpoles compared to controls and the other brain regions. Through molecularly profiling active neurons using phosphoTRAP, I identified proopiomelanocortin (POMC), a precursor polypeptide with multiple post-translational subunits, as enriched in active neurons during aggression. Through pharmacological manipulations with the subunits of POMC (aMSH, ACTH, and B-endorphin), I found that a B-endorphin antagonist significantly increased aggressive behavior in tadpoles. Overall, this project is advancing understanding of how aggression is regulated in the juvenile brain.

F29 Fear contagion in fish: the role of oxytocin and the orthologous autism gene *Shank3*

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Fear contagion is the social transmission of distress and is considered an ancestral form of empathic behaviour, which comprises the recognition of another's negatively valenced distress state and ultimately drives prosocial interaction. This relies on the cognitive processing of information from others and the motivation to approach them. Yet, these components are also susceptible to anxiety, which is by definition effective when local threats are inferred without being directly perceived, such as via the distress of others. Based on this conceptual framework, we first explored the phenotypic structure of motivational, cognitive and anxiety components in wild-type zebrafish, revealing generalized effects on social and non-social contexts, and links between them (*r*

> 0.4). Single-nucleotide polymorphisms in neuroplasticity and reward-pathway genes were implicated across components, but dominant effects were also exhibited on anxiety by the oxytocin gene and on motivation by *Shank3*, a leading autism-pathology gene. We thus investigated the role of these systems in fear contagion, using video-playbacks and kinematic analysis to quantify distress transmission, its recognition as a valenced state (compared to a neutral state) and prosocial response (i.e. approach despite local risk). Using genetic models, the oxytocin pathway was found necessary and sufficient for contagion, recognition and the approach of distressed others, while mutation to the *Shank3* gene elicited deficits across these phenotypes, similar to those in humans and other mammals. Our findings demonstrate that components of distress transmission implicate shared and unique mechanisms, of which certain key neurophysiological and neuropathological modulators have conserved functions on fear contagion in fish.

F30 Serotonin receptors 5HT1A and 5HT3 function in territorial and paternal behaviors: Utilizing organismal pharmacological methods to ascertain behavioral functions

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The Puerto Rican coquí, *Eleutherodactylus coqui*, is a frog that undergoes direct development and exhibits complex social behaviors including territoriality and paternal care. This investigation was an organismal pharmacological study that examined how the serotoninergic receptor systems $5HT_{1A}$ and $5HT_3$ function in territorial and paternal behaviors. Adult territorial, silent (non-territorial), and paternal male coquí frogs were injected (IP) with either one of two selective serotonin agonists, $5HT_{1A}$ (8-OH-DPAT) or $5HT_3$ (SR 57227A).Results indicated that activating $5HT_{1A}$ significantly inhibited territorial behavior in territorial males including decreasing the number of advertisement calls, inhibiting encounter or aggressive calling, submissive male postural displays, and some loss of territories. Silent males did not emit advertisement or encounter calls, did not display any aggressive behaviors, but did significantly move into new areas (with no other conspecifics) more than territorial or paternal males. Paternal males significantly increased numbers of both advertisement and encounter calls, increased movement around nest site, decreased egg brooding and guarding time, increased egg consumption, and increased interactions with conspecifics. Results indicated that activating $5HT_3$ in territorial males significantly increased both advertisement and encounter calls, displayed increased physical interactions, and movements. In silent males, there was no significant differences compared to controls. Paternal males displayed significantly more advertisement calls, increased numbers of encounter calls and physical contacts with conspecifics. These results display important behavioral differences among 5HT receptor function in this complex anuran social system.

F31 Transcriptomic profiling of brood care behaviour in the shell-dwelling cichlid *L. Ocellatus*

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Unlike most other teleost fish, cichlids recognize, protect, and care for their offspring, a behaviour reminiscent of child rearing by birds and mammals. Shell-dwelling cichlids of the species *Lamprologus ocellatus* (LO) are particularly amenable to a genetic dissection of parental behaviour, as they can be bred easily in the laboratory and consistently perform brood care. In their natural environment, the sandy floor of Lake Tanganyika, as well as under confined laboratory conditions, LO search for empty snail shells, which they clean and manipulate in such a way as to serve as a nest for their offspring (see abstract by Grätsch et al.). The fertilized eggs are sheltered inside the shells and cleaned by the female (see abstract by Parker et al.). After the larvae have hatched, the mother keeps guarding the nest and defends the greater territory, allowing the larvae to safely explore and forage around the shell. Once old enough, the mother expels them to prepare for the next round of breeding. We set out to investigate the gene expression changes that accompany transitions between different behavioural states during the mother's reproductive cycle. Single-cell sequencing of the telencephalon is being used to classify neurons into putative types and compare their state-dependent transcriptional profiles. In a pilot experiment, we could already detect transcriptional signatures of neural differentiation, axon guidance and synaptic rewiring in females actively exhibiting brood care behaviour. Next, we will expand our sequencing throughput and validate the differentially regulated marker genes with hybridization chain reaction (HCR) RNA in situ labelling. Here we will report on our progress on establishing LO as a genetic model system for studying complex social behaviour.

F32 Development of social cognition in cichlids: do offspring respond to parental cues?

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In parental care paradigms, it is clear that the mother and/or father have behavioural and often morphological adaptations that enable caring for offspring. Is it then also necessary for the recipient offspring to develop socially cognitive behaviours early on, to facilitate the parental care strategy? The cichlid species flock boasts a large variety of reproductive strategies, raising and caring for their young from eggs until independent juvenile stages. *Lamprologus ocellatus* is one such cichlid with a particularly interesting parenting strategy, raising larvae in the dark and protective confines of an abandoned snail shell and once fry emerge, guarding them from passing predators. We observe the developing larvae to determine whether they actively respond to social cues provided by the parents and additionally replace the parents with simplified cues. We have developed a 5-camera set up containing a customized 3D printed shell which facilitates filming the larvae within and surrounding the shell. We are using object detection and tracking in these challenging environments to automate data collection and analysis to answer two relevant questions. Firstly, what drives the larval cichlids to switch from shell-confinement to free-swimming in the open water? In addition, once freely swimming what mechanism motivates these larvae to remain close to their parent's shell for continued protection? Thus far, we see the mother provides essential care to the passively recipient offspring in the shell and little evidence for active response to her presence from the larvae. Once emerged however, the larvae only have interest in their home shell in the presence of a parent, ignoring the shelter when parents are removed.

F33 Does enrichment of the breeding environment have an impact on the emotional state of the common cuttlefish *Sepia officinalis*?

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Enrichment of the breeding environment is of great importance to allow the animals to express their natural behaviours and thus to provide them with welfare conditions. The housing conditions of laboratory animals must therefore be adapted to each species. Since 2013, the European Directive 2010/63/EU requires the welfare of cephalopod molluscs to be respected, but paradoxically this regulation does not provide precise housing conditions, as is the case for rodents, or even a tool for assessing their welfare. However, it has been shown that enrichment of the breeding environment has an impact on growth, learning, memory, visual perception and colour changes in cuttlefish. Cuttlefish have a skin covered with innumerable pigmented and contractile cells controlled by a developed nervous system (size/weight ratio comparable to that of vertebrates), which allow them to change their colour and body pattern instantaneously. Our recent work has highlighted emotional indicators in *Sepia officinalis* based on colour and pattern changes (ETHiCS project: ANR-18-CE02-0022). Our study focuses on the effect of enrichment of the breeding environment on the emotional state of cuttlefish. Cuttlefish were placed in a physically, socially and nutritionally enriched environment, or in an impoverished environment without enrichment. Changes in colouration indicative of their welfare were observed and correlated with the quantity of monoamines present in the brain. These results show for the first time the effect of the breeding environment on the welfare of cuttlefish.

F34 **Individual consistency in the stinging behaviour of honeybees**

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Honeybees act together as a collective to defend their colony against large vertebrate intruders. To do this, bees release alarm pheromones upon detecting an intruder. This triggers the conspecifics into a frenzy of attack: they head-butt, pull its hair, bite or sting the intruder...anything they can do to deter it! However, attacking is not without risks. In particular, stinging can result in the death of the bee if the stinger gets attached to the skin of the vertebrate intruder. To avoid the loss of colony workforce, we hypothesize that only certain bees commit to defence. Hence, we ask the question of whether individual bees are consistent in their aggressive and stinging behaviours. We use a standardised assay to test for aggressive behaviour and measure stinging responsiveness of every individual bee. On repeating the test in a number of consecutive trials, we can measure whether individuals remain consistent in their decision to attack. Thus, we can quantify for variability at an individual level. Identifying whether individuals are consistent or random in their defensive behaviour helps us to determine how the collective creates an efficient defence. In addition, it opens the door to the identification of the neural basis for aggressiveness via comparative studies.

F35 **Innate behaviors change with ambient light in old and new-world mice**

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Innate behaviors triggered by the appearance of threat and prey are essential for an animal's survival. Aversive and orienting behaviors do not require learning and the underlying brain circuitry is established early in life, making them a valuable model for the neuro-ethological study of sensory-motor transformations. In nature, predators and prey are encountered under a variety of light conditions that are associated with distinct levels of risk and opportunity. However, most studies are performed under standard indoor lighting conditions, and it is unknown how changes in ambient light levels affect predator avoidance and prey capture behaviors. Here we have systematically examined the innate responses of new (*Peromyscus*) and old-world (*Mus*) mouse species to the presentation of visual stimuli that mimic predators or prey under daylight and moonlight conditions. We found both general and species-specific adaptations to the different light levels. For example, looming stimuli mimicking an attacking predator induced a shorter onset and more vigorous reaction in all species under moonlight conditions. On the other hand, prey-mimicking and non-threatening visual stimuli triggered a variety of behaviors across light levels that differed between species. Finally, some pre-stimulus activity was predictive of which behavior an animal would produce in response to threat in a light-specific manner. Taken together, we show that both new and old-world mice adjust their behavior to the ambient light conditions. These findings indicate that light-dependent innate behaviors are an excellent model to investigate the neural mechanisms that enable animals to flexibility adapt behavior to their surroundings and context.

F36 **Hypothalamic galanin neurons modulate stress in zebrafish larvae**

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Stress is a physiological phenomenon fundamental for survival, and its dysregulation is associated with severe types of mental illness, such as anxiety and depressive disorders. Although decades of studies have identified the main stress axes, the neuronal circuits underlying their modulation are not fully understood, as is our comprehension of the mental disorders associated with them. We found that different stressors activate a subset of neurons in the zebrafish hypothalamus producing the neuropeptide Galanin. Ablation of Galanin neurons leads to hyper-responsiveness to stress and increases activity of cells producing corticotropin-releasing-hormone, the main known neuronal population initiating stress responses. Surprisingly, we found that the activity of Galanin neurons is tightly regulated by the neuropeptide Galanin through a self-inhibitory loop. This control system likely modulates a balance of inhibition over downstream stress-promoting neurons, preventing their activation in normal conditions, and their overactivation when stressors are present. Our results provide new insights into hypothalamic neuronal circuits modulating stress and reveal that Galanin neurons in the preoptic area of zebrafish are central players in modulating behavioral and endocrine responses to stress.

CHEMICAL SENSING II

G10 Mating induced attraction to oviposition-related odors in the yellow fever mosquito *Aedes aegypti*

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The yellow fever mosquito *Aedes aegypti* is a prolific disease vector with explosive population dynamics. Its ability to thrive in urban environments lies in the unique ability of female *Ae. aegypti* to seek out standing pools of water to lay their desiccation-resistant eggs. Of note, females are highly attracted to the scent of decomposing botanical matter in water. However, the molecular and cellular basis of how the drive to seek out oviposition sites is guided by the mosquito sense of smell and how this behavior is mediated by mating is unknown. To gain insight into the olfactory preference of female *Ae. aegypti* for oviposition sites, we established a two choice behavioral assay that quantifies attraction to odors emitted by fermenting Bermuda hay, a potent oviposition attractant. Mated and blood fed females of different *Ae. aegypti* strains were more attracted to hay infusion odor than water alone. The preference for hay infusion was not observed in unmated blood-fed, nor mated unblood-fed females. This indicates oviposition odors are only attractive when signals conferring mating status and feeding status converge. Previous studies have revealed that mating-induced signals can come from peptides carried in male ejaculate that originate from the male accessory gland. To test if oviposition odor seeking is modulated by such peptides, we are evaluating the role of male accessory gland proteins and candidate receptors that may mediate such mating-induced shifts in oviposition site search using genome-engineering and heterologous expression. This research will improve our understanding of the triggers of oviposition site search behavior and catalyze development of novel approaches that target this important aspect of female mosquito reproductive biology.

G11 Chemosensory responses in larval malaria mosquitoes

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The mosquito Anopheles gambiae, a major vector of malaria, relies on its sense of smell to locate humans. While recent advances have improved our understanding of olfaction in adult female mosquitoes, larval sensory systems remain understudied. An. gambiae larvae have only ten cells expressing the odorant receptor co-receptor (Orco) in their antennae and five in their maxillary palps (Riabinina et al., 2016). The An. gambiae larval olfactory system could thus serve as a simple and genetically amenable aquatic olfactory model, but suitable behavioural assays are lacking. We have developed a novel assay to investigate chemosensory responses in mosquito larvae. In this assay, behavioural avoidance or attraction in response to chemicals is detected as increased or decreased locomotor activity, respectively. Using this setup, we characterised larval responses to different concentrations of soluble compounds predicted to bind odorant receptors. We found that most test compounds elicited repellent responses in the larvae, while familiar food-associated stimuli were attractive. These experiments shed light on the principles of olfactory coding and behaviour in mosquito larvae, and establish a basis for future studies that will employ transgenic larvae to disentangle the molecular basis of olfactory attraction and repellence.

G12 Olfactory cues and experience dependent preferences guide foraging behavior in leaf cutting ants *Acromyrmex ambiguous*

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Leaf cutting ants from the genus Acromyrmex are considered native plagues in forestal commercial plantations, mainly affecting willow and poplar plantations in the Delta del Paraná region in Argentina. Identifying sensory cues and understating the mechanisms that guide foraging behavior in this species will provide tools for sustainable plague management in plantations. For that aim we started a project that was first directed to determine preferences among native plants by ants Acromyrmex ambiguus. After extensive foraging tests based on field and laboratory quantifications we selected the most and the least preferred plants species that were "Sen del Campo" and "Anacahuita", respectively. Using these two plant species we performed a series experiments to determine the sensory modalities that guide the foraging preferences. By individual and collective behavioral assays we found robust evidence that acceptance and rejection are based on plant odors rather than on contact cues. Volatiles analysis of both plants revealed and number of differential compounds which support that plant identification might depend on olfaction. Finally, we performed a series of experiments aimed at determining whether these preferences are innate or might be modulated by experience. Strikingly, the results obtained until now based on individual preferences, show that ants invert their initial foraging preferences, after they have been forced to collect from the non-preferred plant when it was the only one option available during ten days foraging.

G13 A dual role for prostaglandin F signaling in hormonal and pheromonal signaling in cichlid fish

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Reproduction requires detecting cues that indicate species, sex, and status of potential partners. Animals' signals reflect their internal state, while perception of this information is biased by the reproductive status of the receiver. However, the mechanisms that modulate sending and receiving these signals are unclear. We take advantage of reproductive behavior in the cichlid fish *Astatotilapia burtoni*, which is genetically manipulable and exhibits quantifiable behavioral routines. Progestin and prostaglandin F_{2a} (PGF) have been previously implicated as key signals that convey fertility status from reproductive tract to brain in many vertebrate species. PGF injection rapidly triggers naturalistic spawning behavior. Conversely, CRISPR-mediated deletions of either the progestin receptor (*PR*) or the PGF receptor (*Ptgfr*) result in a complete abolition of female spawning behaviors. These and other results suggest a model in which PR modulates transcription of *Ptgfr* and increased sensitivity to PGF. PGF in turn activates Ptgfr in key regions of the brain to rapidly drive spawning behavior after ovulation. Furthermore, fertile female cichlids release a metabolite of PGF into the environment, attracting reproductive males. Through single-cell transcriptomics, we identify genes essential for specific channels of olfaction; silencing ciliated olfactory neurons abolishes attraction to this cue and subsequent reproduction is disrupted. We identify an olfactory receptor activated by this pheromonal signal, and delineate its evolutionary history. Thus, our work provides insights into multiple pathways by which hormones regulate social interactions.

G14 A simple method for odor discrimination using an isolated locust antenna

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Rapid and accurate detection and identification of chemical odors is important in many natural, as well as technological, applications. As the human sense of smell is insufficient to meet these challenges, many efforts have been dedicated to the development of artificial olfaction devices (e-noses). These, however, are still far from successfully competing with biological (animal) sensors. A highly effective solution, therefore, could be offered by a hybrid bio-technological device that can detect and classify odors. As a first step towards this goal, we report here on the integration of the peripheral olfactory system, i.e. the antennae, of the desert locust (*Schistocerca Gregaria*), in conjunction with an electroantennogram (EAG) system, for quick and simple recording of electrophysiological responses to a range of odors. The performance of our discriminator was evaluated via testing it with a variety of odorants at different concentrations, as well as with different mixtures of odorant pairs. Additional characterization was achieved by examining the responses of different antennal segments and different stimulus durations. The sum of responses of individual olfactory receptor neurons (ORNs) in the locust's antennae creates a sufficiently complex pattern to enable consistent and robust discrimination of odorants, in conjunction with the aid of artificial intelligence tools. Our findings confirm previous reports of ON and OFF neurons participating in the response. We also found that the odorant concentration had little influence on the discrimination abilities. Our findings, together with the simplicity of our methods, make this study highly promising for further development of a bio-hybrid system for odor detection and identification.

G15 Functional study of the queen pheromone receptor OR11 in honey bees (within the genus *Apis*)

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Honey bees, fascinating eusocial insects, employ a rich repertoire of pheromones to ensure intraspecific communication. Although bees' olfactory system has been extensively studied, peripheral processes are still poorly known, like the sensitivity and response spectra of individual ORs (olfactory receptors). This is mainly due to difficulties to record activity from individual OSNs within honeybees' sensilla that contain as many as 35 OSNs. To overcome this problem, we chose to express honeybee ORs within the empty neuron system of *Drosophila melanogaster and use Transcuticular Calcium Imaging to record OSNs.* In this study, we focus on the OR11 present in the genomes of four different species within the genus Apis: *A. mellifera, A. dorsata*, *A. florea* and *A. cerana*. The *A. mellifera* OR11 was shown to respond specifically to the 9-ODA, the major component of the queen pheromone. The 9-ODA is involved in the attraction of drones toward queens during reproduction in the all the Apis species. We chose to study the OR11 orthologs responses to 9-ODA and analysed their specificity by screening few panels of odorants. We observed that *Amel*OR11, *Ador*OR11, *Acer*OR11 and *Aflor*OR11 primarily responds to 9-ODA. Nevertheless, we also recorded lower responses to different odorants for AmelOR11, AcerOR11 and AdorOR11such as trans-2-hexenoic acid and 2-oxovaleric acid. Dose-response analyses show that these are secondary ligands. By examining the of antennal lobe males *A. mellifera* using *in vivo* calcium imaging, we also showed responses to trans-2-hexenoic acid in the 9-ODA-tuned macroglomerulus 2. This work demonstrated that the queen pheromone olfactory receptor OR11 present in Apis species is highly tuned to 9-ODA but also to others ligands with similar chemical conformations.

G16 Functional significance of increased olfactory sensory pooling in a drosophilid specialist

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How nervous system adaptations confer species-specific behaviours is largely unknown. *Drosophila sechellia* is a powerful model for comparative neurobiology as it is closely related to *D. melanogaster* but has adapted to a distinct ecological niche, feeding and breeding exclusively on the noni fruit of Morinda citrifolia. Noni emits odors that attract *D. sechellia* but not *D. melanogaster*. We previously identified olfactory receptors that are required for noni attraction, including Or22a and Or85b/c. Notably, the number of olfactory sensory neurons (OSNs) expressing these receptors has increased three-fold in *D. sechellia*. However, the functional significance of such OSN population expansions is unknown. Using new genetic tools in *D. sechellia*, we have performed comparative anatomical and physiological analyses of the olfactory circuitry of *D. sechellia* and *D. melanogaster*. While Or22a and Or85b/c OSN populations are unique in their dramatic expansion in *D. sechellia*, the number of partner projection neurons (PNs) for these pathways are conserved between species. Using calcium imaging, we observed that, despite the greater sensory pooling, Or85b/c PN sensitivity is not enhanced in *D. sechellia*. However, *D. sechellia* Or85b/c PNs exhibit much less short-term depression to pulsed or long-lasting odor stimuli. Such reduction in short-term depression is specific to sensory channels where OSN number has increased. These observations suggest that OSN number increases enhance host fruit location in *D. sechellia* by sustaining PN responses, synergizing with sensitivity increases conferred by receptor re-tuning. Our ongoing work compares the habituation between species to explore the functional link between OSN number increase and persistence in odor-plume tracking.

G17 Compounds without borders: a novel paradigm for quantifying complex odors and responses to scent-pollution in bumblebees

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Bumblebees are critical pollinators whose populations are drastically declining. Successful foraging improves colony fitness, thus understanding how anthropogenic influences modulate foraging behavior may aid conservation efforts. Odor pollution can have negative impacts on bumble- and honey-bees foraging behavior. However, given the vast array of potential scent contaminants, individually testing pollutants is an ineffective approach. The ability to quantitatively measure how much scent-pollution of a floral-odor bumblebees can tolerate would represent a paradigm shift in odor-pollution studies. Current statistical methods derive the dimensions of an ‘odor-space' from the odorants within a dataset; therefore, when the dataset is modified the odor-space itself is reconstructed. In this way statistical methods such as principle components analysis (PCA) or non-metric multidimensional scaling (NMDS) have excellent descriptive power, but are less effective at prediction This study presents an alternative method of analyzing complex odor blends based on the encoding properties of insect olfactory systems. This "Compounds Without Borders” (CWB) method represents odors as a vector in a multidimensional space representing the functional group and carbon characteristics of their component odorants. The dimensions of this space are independent of the data described within it. These vectors allow the angular distance between any two odors to be calculated including a learned odor and its polluted counterpart. Data presented here indicate that CWB-angles are capable of both describing and predicting bumblebee odor-discrimination behavior: odor pairs with angular distances in the 20-29° range are generalized, while odor pairs over 30 degrees are differentiated.

G18 Social modulation of food odorant processing in the locust antennal lobe

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Flexibility in social foraging behavior allows animals to maximize foraging success in nutritionally unpredictable environments. The desert locust *Schistocerca gregaria* exhibits one of the most extreme examples of this flexibility. Usually, solitarious locusts populate sparse landscapes at low densities and forage alone. However, once conditions are suitable, they undergo a phase change and become gregarious. The transition to group foraging strongly impacts the type, quality, and quantity of information available to individual animals. In addition to personally acquired evidence, gregarious locusts have access to a plethora of social information, allowing them to integrate socially derived clues on the location and quality of a food source. How does the social context, such as the smell of conspecifics, guide animals in their foraging campaigns, and are there differences between the two phases? Here, we investigate the early olfactory processing of food odorants in the presence and absence of the colony smell via calcium imaging of antennal lobe projection neurons in gregarious and solitarious animals. We demonstrate that a simulated olfactory group context increases the overall magnitude of projection neuron activity to food odorants in gregarious animals. This social modulation is phase-dependent and does not occur in solitary animals, suggesting it to be a potential adaptation of the olfactory system to facilitate foraging in a group.

VISION AND PHOTORECEPTION II

I23 Retinal mosaic contribution to spatial and spectral interactions among photoreceptor axons in the lamina of *Papilio Xuthus*

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Color discrimination of *P. xuthus* butterfly is remarkable, and its early visual system is accordingly complex. The compound eyes are built of ommatidia with nine photoreceptors (PR) belonging to six spectral classes, forming three distinct ommatidia types. The ommatidia are randomly distributed in a ratio of 2:1:1 for Types I, II, and III, respectively. The PRs show spectral opponency, depolarizing or hyperpolarizing to different wavelengths of light. Recently, we reported that an inhibitory synaptic network in the first optic ganglion (lamina) shapes the spectral responses of PRs. The network presumably tunes the PRs' spectral sensitivity for color detection. The synapses inside single ommatidia explain the opponency evoked by on-axis light stimulation. However, ~20% of inter-PR synapses are inter-ommatidial. To investigate the inter-ommatidial synaptic network, we measured the spatio-spectral response of PRs and lamina monopolar cells (LMCs) in the lamina using equiquantal monochromatic flashes. We recorded the spectral responses of penetrated cells at ~50 different angular locations. The response *amplitude* varies with distance from the center and the *shape* with direction from the center, indicating that the neighboring ommatidial composition is important for spatial pre-processing. We suggest that the synaptic interactions between the PRs can enhance edge detection, increase color contrast, and adjust the general sensitivity of PRs for a wider dynamic range in the lamina.

I24 Adaptions of the peripheral visual system to dim light in hawkmoths (*Sphingidae*)

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Dim light creates many challenges for color vision. Both the amount of light and spectra drastically change, being characterized by dimmer, more long-wavelength (LW) shifted light. Hawkmoths (Sphingidae) have evolved several neural adaptations enabling them to use vision for foraging and color processing at extremely low light levels. Here, we investigate if the peripheral visual system via opsin sensitivity tuning may act as a potential adaptation to use LW-shifted dim light. Using visual modeling, we first predict chromatic and achromatic detection of a variety of flower colors when viewed under daytime and nighttime conditions. We find that floral chromatic detection is significantly better for a green receptor (530 nm) in both day and nighttime conditions, but a long wavelength shift (630 nm) significantly improves detection of achromatic cues. However, when we limit the chromatic analysis to flowers specifically adapted to nocturnal hawkmoths, we find that a 630 nm receptor performs as well as a 530 nm receptor in nighttime conditions, meaning a LW-shifted receptor maintains chromatic detection of relevant stimuli in dim light. We then characterize the spectral sensitivity of LW sensitive opsins for diurnal and nocturnal hawkmoths via RNA-seq and in vitro heterologous expression. We show that diurnal species have retained a green-sensitive LW opsin, whereas preliminary data suggest a LW shift at least in some nocturnal species. Combined, this indicates that while chromatic cues are better processed by

the green receptor typical of many diurnal insects, LW-shifted sensitivity in nocturnal species can improve flower detection in dim-light. Consequently, our studies suggest that opsin tuning acts as an adaptation to dim light vision in some nocturnal hawkmoths.

I25 Here comes the Sun: Effects of abrupt and gradual changes in light intensity over a 24h period in a nocturnal ground beetle

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Most animals adjust their activity levels in response to changes in the environmental light intensity. In many insects, light intensity is a major regulator of their circadian rhythms and sleep patterns (reviewed in Helfrich-Förster, 2019; Mazzotta *et al*., 2020). Typically, experiments use alternating light and dark phases, with abrupt transitions between the two. However, environmental changes in ambient light intensity due to sunrise and sunset are gradual and abrupt changes are often signals of disturbance or danger. Little is known about the differences in the effect of gradual and abrupt transitions upon insect activity patterns. Here, we explore this question using the ground beetle *Nebria brevicollis*, which is nocturnal and found year-round in the leaf litter of Northern temperate forests. We observed beetles for 24 hour periods under four different lighting conditions: abrupt light/dark (A-LD), gradual light/dark (G-LD) light/light (LL) and dark/dark (DD). We developed a setup, using a 6-wellplate to monitor 6 beetles at once with an infrared camera along with dedicated software for flexible experimentation, Bux Recorder, allowing detailed logging of both behaviour and environment. Videos were subsequently tracked using TRex, a tracking software (Walter & Couzin, 2021). I will present findings of *Nebria brevicollis* responses to abrupt and gradual transitions between light and darkness. We expect our results to inform a more naturalistic, ecologically relevant approach to studying transitions in ambient light intensity, including between sleep and wakefulness in the insect brain.

I26 Prey capture and escape behaviors of male and female Neohelice crabs to moving objects are differentially affected by the level of starvation

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Intrinsic and extrinsic factors alter the behavioral outcome to specific stimuli. When a crab sees a small object moving on the ground it can react in different ways: a) ignore the stimulus (NR: no response), b) freeze (FR: freezing response), c) move away or display a defensive reaction (AR: avoidance response), and d) run after to capture the object (PR: predatory response). We have previously shown in males that the probability of eliciting each type of response depends on the size of the dummy stimulus. Here we investigated whether the sex and the level of hunger affect the election among these response. In a first experiment, we investigated responses to dummies of crabs along 22 day of starvation. Males showed a higher PR probability than females while females presented higher AR and FR probability than males. Along 22 days of starvation there were no differences in the behavior probabilities for the medium dummy in both sexes. However, there were large differences with the small dummy, for which females and males increased PR and decreased FR along days. To test if the increment of PR and reduction of AR were due to the food shortage, in a second experiment, we compared the behavior of males regularly fed with that of starved males along 15 days. As in the previous experiment, starved males increased the PR along days while fed males did not. The AR, FR and PR are initiated at different crab-dummy distances, being the PR and FR initiated at shorter distances than the AR. However, no effects of sex or starvation on response distances could be disclosed. Our main results show that: a) males display stronger PR than females; b) hunger increases the PR probability, mainly to smaller stimuli, without affecting response distances.

I27 Kinetically matched head and eye rotations are synchronized to stabilize visual scene in freely moving mammals

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Vestibular and image-feedback driven compensatory eye rotations are known to reduce image blur caused by movements of the head. However, during pursuit, there are multiple objects for the visual system to track. Under these conditions, it is not known how compensatory eye and head movements are coordinated, nor is it known what aspect of the visual scene is stabilized or how retinal specializations, like the area centralis, are moved around. Here we tracked both head and eye rotations in ferrets pursuing an erratically moving target. We show that when the animal made turns as it pursued the target, the head was not held in a constant stationary position on the shoulders. Instead, a series of active, step-like head rotations (mean ± SD amplitude, 30.42 ± 16.78°, n=127 saccades from 2 animals) were made, with each head turn associated with a precisely coordinated compensatory saccade (mean ± SD amplitude Left and Right eye respectively, 12.71 ± 5.15°, 12.31 ± 6.38°, n=127 saccades from 2 animals). Both rotational amplitudes and velocities of individual head and eye saccades were matched and scaled together, enabling a reduction in rotation-induced motion blur across a wide range of head rotation amplitudes (range 6.22–98.10°, n=127 saccades from 2 animals). The compensation preferentially stabilized image background motion, and not motion of the target, increasing target 'motion contrast' (target motion / background motion) in 86.7% of saccades. While the saccades moved the area centralis towards the target, precise target fixation was observed in only 13% of the saccades. Head and eye rotation amplitude matching was also measured in tree shrews, suggesting a generalized and flexible mechanism for image stabilization during self-induced motion in mammals.

I28 CompoundRay: simulating insect vision accurately and fast

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Compound vision represents a potential wealth of insights into the kind of small-scale, low-power visual processing that insects perform routinely, allowing them to successfully navigate complex visual environments with ease. To date, `Compound Eye Models' (CEMs) have largely explored the effects of individual elements of compound vision such as spectral sensitivity, field of view or angular resolution on behaviour. Yet, the roles of shape, overall structure and how these variables interrelate have been largely overlooked due to modelling complexity. However, modern real-time raytracing technologies are enabling the construction of a new generation of computationally efficient, high-fidelity CEMs. CompoundRay is a new open-source CEM software that leverages modern graphics processing hardware to accurately render the visual perspective of the compound eye at thousands of frames per second, supporting ommatidial arrangements at arbitrary positions with per-ommatidial heterogeneity. Using CompoundRay, we are able to thoroughly and efficiently explore the design-space of compound vision systems at an unparalleled scope.

I29 The neuroethology of distributed vision in chitons and scallops

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Vision is usually thought to be inextricably linked to cephalization; however, certain species of invertebrates deviate from this canonical association by having visual systems that are composed of numerous photoreceptive

organs distributed across their bodies. In contrast to cephalized vision, we know comparatively little about the neuroanatomy and behaviors associated with distributed vision. To investigate different modes of distributed vision, we explored the neuroanatomy and visually-mediated behaviors of the chiton *Acanthopleura granulata* and the bay scallop *Argopecten irradians*, which have distributed visual systems composed of hundreds and dozens of eyes, respectively. Using behavioral trials, we found that *A. granulata* demonstrate a visual acuity of 6° and respond to polarization defined stimuli. Using neural tracing, we found that the optic nerves of *A. granulata* form a distributed visuotopic map along the lateral neuropil which is a continuous neural layer that circumnavigates the body. Using behavioral trials, we found that *A. irradians* demonstrate spatial vision (i.e. the ability to locate visual cues) by extending their sensory tentacles towards and tracking visual stimuli as small as 5°. Using neural tracing techniques, we found that *A. irradians*' optic nerves innervate the cortical surfaces of the lateral lobes of the visceral ganglion to form discrete somatotopic maps. These two animals with different types of distributed visual systems use distinct and novel approaches for visual integration and processing. *A. granulata* appears to construct a decentralized visuotopic map to support decentralized visual-motor circuits, whereas *A. irradians* appears to construct a centralized spatiotopic map to guide complex decentralized visually-mediated behaviors.

I30 Vision in drosophilids from disparate visual landscapes

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Drosophilids are ecologically successful flies, and one of the most intensively studied animal groups in history. But findings from just one species are often generalized to the entire, diverse group---or even flies as a whole. Like most flying insects, visual navigation is a fundamental aspect of their success, but different fruit fly species can inhabit regions that are strikingly varied. To determine how vision and flight control are linked to the visual aspects of native environment, we compared the optics and steering responses in three species: Drosophila melanogaster, a cosmopolitan fly originally endemic to habitats with dense vegetation, Zaprionus indianus, which flies under fig trees that potentially induce heavy dorsal optic flow, and Drosophila mojavensis, which flies in arid regions which may generate mostly ventral optic flow. Some visual traits were common to all three species, but we found distinctive strategies in optical layout, steering during optical flow, and neural responses under dim light. These results highlight aspects of fruit fly vision that do not generalize across species, but are selectively tailored to the visual elements of their natural habitat.

I31 Development of the binocular visual field in a diurnal precocial rodent, the *Octodon degus*

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Mammals exhibit the highest degree of binocularity among vertebrates. However, the shape, orientation and extent of the binocular portion of the visual field is highly variable among them, ranging from a narrow, dorsal longitudinal band typical of lagomorphs and rodents, to a wide, frontal binocular field in primates. Comparative studies have also shown that the convergence of the orbits is a direct predictor of the binocular field extent. Visual field and orbit orientation are highly plastic and display strong correlation with the animal´s visual ecology and behaviour. However, the developmental mechanisms that support the phylogenetic plasticity associated to these traits remain largely unexplored. In this study, we described for the first time the ontogeny of the visual field and orbit orientation using as a model a diurnal precocial rodent endemic to Chile, the *Octodon degus*. Degus are born eye-opened and display visually guided exploratory behaviours soon after birth. We performed visual field and orbit convergence measurements during the first postnatal month (P5, P9, P14 and P30) and in adults (at 6 - 12 months of age). As other rodents, adult degus displayed a longitudinal band of binocular field with a maximal azimuthal extension of ~60°. Surprisingly, at P5 the maximal azimuthal extension reached only

~20° and progressively expanded at subsequent stages. Likewise, the angle formed between the orbits progressively converged in parallel to the development of the binocular visual field. Our results showed that the visual field of degus matures postnatally when de animal is visually active, which would open the possibility for experience-dependent developmental plastic transformations that in evolutionary time may contribute to the present phylogenetic diversity.

I32 A three-photon head-mounted microscope for imaging all layers of visual cortex in freely moving mice

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Miniaturized head-mounted two-photon microscopes have enabled imaging of activity from fluorescently labelled neuronal populations in the upper cortical layers with single cell resolution in freely moving rodents. Three-photon excitation (3PE) can considerably extend the imaging depth possible in scattering tissue by utilizing longer wavelengths, that decrease excitation light scattering and eliminating the generation of out-of-focus fluorescence. However, 3PE-based head-mounted microscopes have so far been too physically restrictive to take advantage of mouse-based molecular tools. Here we built a 2 gram, 3PE-based microscope, capable of imaging activity from neuronal populations from all cortical layers in the freely moving mouse. This tool is equipped with a z-drive, enabling remote focusing through the whole cortical depth without interfering with the animal's behavior, and resilient miniature detectors that enable imaging in a fully lit environment with high sensitivity. We use it to show that neuronal population activity in cortical layer-4 and layer-6 was differentially modulated by lit and dark conditions during free exploration. Together this new microscope enables studies of cortical computation in freely-moving animals over a large range of natural behaviors.

I33 Resplendent reflections: Mueller matrix characterizations of circularly polarized reflectance from jewel scarabs

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Circularly polarized light (CPL) reflections are rare in nature. Only a few animal groups - most notably certain stomatopod crustaceans and beetles in the family Scarabaeidae - are known to reflect CPL from incident unpolarized light. Many arthropods detect linearly polarized light, but stomatopods are currently the only group known to have a mechanism to discriminate CPL. It remains unclear if any scarabs can use CPL. To explore this, first we characterized how CPL signatures appear in the natural environment. Using a spectropolarimetric reflectometer to measure the Mueller matrix elements of 50 species of beetles' elytral surfaces, we found that all but one species were strongly left-handed circularly polarized (LHCP), and only one species, *Chrysina resplendens*, switched from LHCP to right-handed circularly polarized (RHCP) reflectance depending on wavelength. This wavelength dependent switch has previously been suggested to occur as a result of their overall lower degree of polarization (i.e. the switch from LHCP to RHCP is not functionally significant and is simply oscillating around zero), so we measured variation among 15 specimens of *C. resplendens* to demonstrate the robustness of the LHCP vs. RHCP optical signature at a given wavelength. Also, by taking Mueller matrix measurements that average over the entire elytral surface at normal incidence, we can compare our results with those obtained from ellipsometry methods and with those that characterize handedness based on viewing the beetles through polarizing filters in order to gain new insights into any ecological and neurobiological relevance of these signatures. Next steps include examining scarab eyes for birefringence and using electrophysiological methods to test whether they respond to CPL stimuli.

<u>I34</u> Connectivity in the optic lamina of two stomatopod superfamilies

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The optic lamina is the first step in visual information integration past the photoreceptors in crustaceans and insects, containing the first synaptic connections between photoreceptors and first-order interneurons, including monopolar cells. The lamina circuitry in stomatopods is especially interesting as they likely do not use opponency and instead use a novel processing method to interpret visual input. Using Serial Block-Face Scanning Electron Microscopy, we have reconstructed photoreceptor terminals and lamina interneurons in all stomatopod cartridge types (midband and hemisphere) and examined differences between the hemispheres of two major superfamilies showing different visual ecologies (Gonodactyloidea and Lysiosquilloidea). Connectivity between photoreceptors and interneurons, as well as cross-cartridge connections, is estimated based on neuron overlap. Neurons are identified based on previous Golgi data, and follow the same general pattern as seen in other crustaceans. The photoreceptors form bulbous terminals in the lamina, following the same overall pattern in all lamina cartridges across all species and eye regions. This termination pattern differs from previous research, due to the new methodology, creating a slightly different circuitry in this neuropil. The photoreceptor terminals themselves appear to be more complex, with many large mitochondria which fill the terminal area, which may have implications for differences between insects and stomatopods, or even crustaceans as a whole. This greater complexity suggests that the stomatopod lamina performs differently in the processing of visual signals than insects, and provides further evidence to the emerging hypothesis that crustacean and insect vision may be more different than previously assumed.

<u>I35</u> Spectral sensitivity and chromatic vision of Australian jewel beetles

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Colour vision is widespread across the animal kingdom and is essential for many behaviours such as finding food or mates. Both the number of photoreceptors and their spectral sensitivity influences colour vision. Whilst research in the field of visual ecology has significantly expanded, we still have limited understanding of how the vast variation in spectral sensitivity we see across the animal kingdom relates to behaviour, ecology and environment. Here, we use electroretinograms to investigate whole eye spectral sensitivity of Australian jewel beetles from four subfamilies with diverse food sources and activity times. Our results indicate that these jewel beetle species have three to four photoreceptors that span a large part of the wavelength spectrum from ultraviolet to red wavelengths (~350 – 640 nm). We detected variation in both short wavelength and long wavelength sensitivity among species. To investigate whether these photoreceptors are used for chromatic vision, we conducted behavioural choice experiments using two jewel beetle species. Preliminary results suggest that both species prefer the ultraviolet light stimulus over the blue, green or red stimuli, regardless of intensity. Preference tests between the remaining stimuli are ongoing but currently suggest no preferences. We will discuss how this variation may correspond to the ecology and behaviour of jewel beetles.

<u>I36</u> Trichromatic retina is highly conserved among tortricid moths

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Leafrollers (Lepidoptera: Tortricidae) are a large family of small moths containing over 10.000 species, many of which are crop pests. It includes both nocturnal and diurnal moths and although much is known about their olfactory system due to the importance of pheromones to control them, there is not much information about their visual system. *Grapholita molesta*, *Lobesia botrana* and *Cydia pomonella* are widespread tortricids fruit pests. The adults have discrete periods of sexual activity that occur before, during and after sunset, respectively.

We wanted to determine if being active at different times of the day and night is associated with differences in their visual system. Spectral sensitivity (SS) was measured with electroretinograms (ERG) and selective adaptation with green, blue and ultraviolet light. SS curves could be fitted with a triple nomogram template which indicated the existence of three photoreceptor classes peaking at 355 nm, 440 nm and 525 nm. The retinae showed clear regionalization, with fewer blue receptors dorsally. No differences among species were found. Intracellular recordings in *Cydia pomonella* photoreceptors revealed three photoreceptor classes with narrow sensitivity, peaking at 525, 440 and 355 nm and a broadband photoreceptor peaking at 525 nm. The blue photoreceptors showed opponent responses in the green, which could be altered with current injection. Flicker fusion frequency experiments showed that the response frequency was similar among sexes and species at around 90 Hz. Our study sheds some light on the relatively unexplored visual system of this economically important insect group.

I37 Temporally-linked visual recognition in insects

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Microsaccadic oscillations in the retina of insects was shown to significantly increase the resolution at which visual inputs can be processed. This implies an ability to detect objects at greater distances than previously thought. However, learning to associate objects with reward or punishment only occurs at specific points in time, usually on close encounters, where the raw visual input might not be similar to that seen from distant viewpoints. In order to transfer what was learned from one perspective to other perspectives of the same object, invariant visual recognition is necessary. We propose a mechanism where neural facilitation in the mushroom body can be used to link visual inputs in time in order to achieve visual invariance. By exploiting spatio-temporal information, scenes containing relevant objects can be associated together. We show empirically that a model of the mushroom body where neural facilitation is used helps to transfer associative learning to other relevant stimuli, and thus detect previously encountered objects from novel viewpoints.

I38 What a bird's eye tells a bird's brain

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Bird retinas are some of the most structurally complex amongst vertebrates, featuring extreme cellular densities alongside specialisations such as six types of cones, oil droplets and sometimes one or even multiple foveas. How do their retinas 'function'? We used high-density multielectrode array recordings to measure spiking responses of 6,000s of retinal ganglion cells in the peripheral chick retina to a battery of visual stimuli modulated in wavelength and time. Across our preliminary dataset of 6000 RGCs, responses were highly diverse. They could be sorted into 27 functionally distinct clusters, showing the following characteristics: First, the vast majority of RGCs were OnOff, "slow", and highly informative about colour. Typically, these RGCs leveraged complex combinations of 'classical' spectral opponency alongside spectrally dependent differences in response-timings. In contrast, a very distinct minority of Off-biased RGCs were essentially achromatic and "fast". The spectral tuning of these fast cells is approximately consistent with the expected spectral tuning of double-cones. We find that already at the population level colour information could be readily read out in several ways. For example, spectrally broad "white" stimuli yielded a high population synchronicity for the Off response, while all "coloured" stimuli, independent of wavelength, instead yielded a relatively increased population synchronicity for the On-response. We tentatively suggest that birds might use a combination of time- and opponency-coding to inform the brain about the spectral composition of the stimulus, while a minority of fast greyscale channels serve contrast vision.

↑UP

I39 Rapid adaptation to new light environments mediated by photoreceptor outer segment plasticity in the developing retina of Atlantic halibut (*Hippoglossus hippoglossus*)

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Colour vision is mediated by input from at least two photoreceptor types, with different visual pigment protein (opsin) expression. Previously, alteration of background light spectrum was shown to rapidly modulate transcript expression (2-3 days) in some fish species. This environmentally-induced plasticity was assumed to underlie a change in the relative density of photoreceptors expressing a given opsin. In this study, Atlantic halibut larvae were exposed to a restricted spectrum (blue, white/control) for one week at the start of eye migration. Quantitative PCR analyses showed that *rh2* (green) and *lws* (red) opsin transcripts were significantly higher (2.1-3.5 fold difference) in retinas from blue-light treated fish compared to the control. In contrast, *sws2* (blue) opsin transcript expression remained unchanged. The densities of cones expressing either SWS2 or longer wavelength (RH2, LWS) opsins were the same across light treatments. However, the outer segments of RH2/LWS expressing cones were 25-30% longer in retinas from the blue light treatment compared to those from the control. This difference was not observed with SWS2 expressing cones. Transmission electron microscopy revealed that lamellar density was unchanged between treatments suggesting that blue light alters the trimming/recycling processes carried out by retinal pigment epithelium cells. Our results reveal a novel mechanism of photoreceptor plasticity that accommodates rapid increases in opsin expression following a change in light background. This mechanism compensates for reduced illumination in a given part of the spectrum and may be ecologically relevant as fish adapt to novel light environments.

I40 Contrast-polarity specific mapping optimizes neuronal computation for collision detection

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Neurons receive information through their synaptic inputs, but the functional significance of how those inputs are mapped on to a cell's dendrites remains unclear. We studied this question in a grasshopper visual neuron that tracks approaching objects and triggers escape behavior before an impending collision. In response to black approaching objects, the neuron receives OFF excitatory inputs that form a retinotopic map of the visual field onto compartmentalized, distal dendrites. Subsequent processing of these OFF inputs by active membrane conductances allows the neuron to discriminate the spatial coherence of such stimuli. In contrast, we show that ON excitatory synaptic inputs activated by white approaching objects map in a random manner onto a more proximal dendritic field of the same neuron. This random synaptic arrangement results in the neuron's inability to discriminate the coherence of white approaching stimuli. Yet, the neuron retains the ability to discriminate stimulus coherence for checkered stimuli of mixed ON/OFF polarity. The coarser mapping and processing of ON stimuli thus has a minimal impact, while reducing the total energetic cost of the circuit. Further, we show that these differences in ON/OFF neuronal processing are behaviorally relevant, being tightly correlated with the animal's escape behavior to light and dark stimuli of variable coherence. Our results show that the synaptic mapping of excitatory inputs affects the fine stimulus discrimination ability of single neurons and document the resulting functional impact on behavior.

I41 Extra-ocular camouflage of flatfish

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Flatfishes exhibit dynamic camouflage by changing the colour of their skin to resemble that of the ocean substrate and avoid predation. It has long been assumed that such camouflage is visually mediated. Here, we used common sole, *Solea solea*, to test whether visual input was required for the camouflage response. Fish

were recorded as they experienced a changing photographic substrate that was either uniform white, or a black and white checkerboard pattern. Common sole responded within minutes with pale colouration to the white substrate, and with mottled colouration, based on the appearance of dark spots of different sizes, to the checkerboard substrate. Following light blockage to both eyes or to the top of the head, the fish continued to exhibit camouflage responses that were similar to those observed when these body regions were not occluded. Thus, dynamic camouflage did not require visual or brain photoreception. Fish were then subjected to the white substrate over a patterned thermal background mimicking that measured from the checkerboard using an infrared camera. The fish responded with mottled skin pattern demonstrating thermal input underlying the camouflage response. Because substrate heat maps of similar magnitude were measured in the near-shore environment, skin thermal sensation could be used by flatfishes to achieve camouflage in shallow waters, when they are most vulnerable to predation.

I42 Defensive shimmering responses in the Asian giant honeybee Apis dorsata are triggered by dark stimuli moving against a bright background

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Giant honeybees, including the open-nesting Asian giant honeybee Apis dorsata, display a spectacular collective defence behaviour – known as “shimmering” – against predators, which is characterised by travelling waves generated by individual bees flipping their abdomens in a coordinated and sequential manner across the bee curtain. We examined if shimmering is visuallymediated by presenting moving stimuli of varying sizes and contrasts to the background (dark or light) in bright and dim ambient light conditions. Shimmering was strongest under bright ambient light, and its strength declined under dim-light in this facultatively nocturnal bee. A. dorsata shimmered only when presented with the darkest stimulus against a light background, but not when this condition was reversed (light stimulus against dark background). This response did not attenuate with repeated exposure to the stimuli, suggesting that shimmering behaviour does not undergo habituation. We suggest that this is an effective anti-predatory strategy in open-nesting A. dorsata colonies which are exposed to high ambient light, as flying predators are more easily detected when they appear as dark moving objects against a bright sky. Moreover, the stimulus detection threshold (smallest visual angular size) is much smaller in this anti-predatory context (1.6o - 3.4o) than in the context of foraging (5.7o), indicating that ecological context affects visual detection threshold.

I43 Visually guided approach and reaching in the Hummingbird Hawkmoth

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Insects, despite their small brains, are capable of performing complex motor tasks. Two such tasks are visually guided approach and reaching towards a target. Both behaviours require conversion of visual cues to a movement in 3D space. This movement should bring the insect or its reaching appendage closer to the target. Hummingbird hawkmoths are equipped with a long proboscis which they use to feed from flowers. Behavioural experiments show that they are able to use vision to approach an artificial flower and use their proboscis to initiate contact with the surface. Recent studies show evidence that they continuously update their proboscis movements on the flower, once initial contact is made, with visual feedback. Therefore they are an excellent example of an insect using vision to control approach and reaching in a computationally efficient manner. In our work we seek to uncover 1) the relevant visual cues and 2) processing methods used in the control strategy that underlies the hawkmoth's approach and initial reach behaviours. In determining the underlying strategy, we hope to develop principles for efficient visually guided reaching in aerial robots. Flower edge motion has been hypothesised as a visual cue to regulate distance between the hawkmoth and a flower, when the hawkmoth is feeding from it. In this poster, we present how this visual cue could be integrated into a vision based control strategy for approach and reaching in an aerial robot platform. We discuss whether this depth cue is sufficient to explain approach and initial reach behaviours.

LEARNING, MEMORY AND COGNITION II

J16 Improved learning and memory retention performance in honeybees using bimodal versus unimodal stimuli, and the future use of multimodality in electroreception research

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The sensory world of bees consists of stimulation by multiple modalities. Alongside the constant mechanosensory stimulation when in flight or in the hive, bees are presented with strong visual and olfactory cues from flowers, as well as floral electric fields, which are detected through the recently discovered sense of aerial electroreception. The proboscis extension response (PER) is a common method for studying learning and memory retention in honeybees, however, despite the importance of multimodal perception, the majority of PER studies have focused only on unimodal stimulation. To rectify this, we compared the effect of different strength unimodal and bimodal stimuli on learning and memory retention performances of honeybees. We start by conditioning honeybee PER to mechanosensory stimulation caused by airflow, then supplement the airflow with an olfactory stimulus. Preliminary data show that bimodal stimulation results in better learning and memory retention than unimodal stimulation. When the mechanosensory stimulus was supplemented by an odour, the proportion of bees that learned the stimulus after four trials increased from 48-65%. These experiments are the foundation for a larger multimodal PER study, in which we will build on the emerging field of aerial electroreception to assess the effects of multimodality on the learning and retention of electrical stimuli. To ensure an ecologically relevant evaluation of electroreception, it must be assessed in conjunction with other sensory modalities and not in isolation. Furthermore, these experiments will be combined with electrophysiology to provide behavioural and physiological evidence to assess the relative prominence and sensitivity of electroreception amongst other sensory modalities in bees.

J17 Has artificial selection for shoal preference in zebrafish driven the evolution of enhanced cognition?

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The social intelligence hypothesis posits that group living generates the selective forces that drive the evolution of enhanced cognitive abilities. This hypothesis has generated a large debate and the available evidence supporting it is still scarce. Experimental approaches that explicitly test their assumptions and predictions are necessary. We have been doing this with zebrafish (*Danio rerio*), a species that has become an established vertebrate model in behavioural neuroscience. We phenotyped a base (F0) population of Tuebingen (TU) zebrafish for sociality using a social preference test. The test consisted in presenting a social stimulus (a video-playback of a shoal of four fish) against a non-social stimulus (a video-playback of a "shoal" of four objects, circles, moving randomly in the same background). We established four F1 selection lines from the F0 by crossing fish with the highest (Shoal preference line), the lowest (Circles preference line), equal (No-preference line), or random (Random preference line) preference for shoal. The selection experiment has now reached the F5 generation with a clear increase in shoal preference of the shoal preference line compared to the other three. We are now testing the learning ability of fish from each population, using reversal and detour learning tasks. We are testing reversal learning with a social stimulus (a video-playback of a shoal of four fish) and for both

females and males. And we are testing detour learning with a non-social stimulus (food) only for females. We will present the preliminary results of the learning tests in the four selected lines.
Project financed by FCT (PTDC/BIA-COM/3068/2020).

J18 How the fly decides: behavioral, genetic, and neuronal analysis of action selection

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We established a new behavioral paradigm in *Drosophila* to ask whether flies use distinct or shared circuits to choose among three advantageous action sequences: grooming, feeding, and courting. Our analysis of wild-type and *FoxP* mutant flies showed unexpected complexity in alternation among behaviors; we are now screening neuromodulatory neurons. Sometimes organisms face conflicting drives or competing sensory stimuli and must select the best response. The brain regions, neural mechanisms, and genetic contributions to perceptual and value-based decision making have been studied in a variety of systems, yet uncertainties remain. *Drosophila* is a valuable system for mapping underlying circuits and genetic contributors. We present flies with pair-wise decisions and demonstrate that they change their selection based on respective drive strength rather than an absolute hierarchy of needs: we observed that flies that are both dirty and hungry change their action selection from grooming to feeding depending on starvation time as opposed to always doing one behavior first to completion before preceding to the next. We established drive conditions such that flies spend approximately equal time performing two competing behaviors, and then analyzed the timing of their actions. As drive strengths became more equal, flies multi-task: for example, some of our dirty and hungry flies will eat and groom simultaneously. We also found that flies will task-switch rapidly, alternating bouts of mate-chasing with grooming or eating. *FoxP* mutations led to increases in bout durations during competing behaviors. To identify circuits that affect decision making, we are using optogenetic approaches to screen different populations of aminergic and peptidergic neurons in our behavior-choice assays.

J19 Identifying natural transitions from goal-directed to habit-like performance during sensorimotor learning in mice

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Animals use different decision processes to efficiently adapt to complex environments. When in a goal-directed mode, animals deliberate in a slow and cognitively demanding process. With time, animals can shift to a more automatic habit mode. The nature and timescale of the transition from goal-directed to habitual performance, however, remains poorly understood. During learning, a challenge to identifying the decision process is that the decision itself is a hidden variable not behaviorally observable. For one, performance outcomes (action rates and accuracy) are often similar when using either decision process. Secondly, in water-restricted (WR) paradigms, an animals' drive is based on a need, linked to survival (hydration), so animals exhibit constantly high action rates and gradually improve accuracy. We hypothesized that by shifting an animals' motivation from a need to a preference, we could use action rate variability as a behavioral indicator of goal-directed performance (highly variable) versus habit-like performance (stable). We leveraged a recent protocol where mice get access to water with citric acid (CA), a non-palatable substance that fulfills hydration. We compared CA mice with mice under WR protocols. All groups were trained in an auditory go/no-go task in which they can obtain plain water. Our data shows that all groups of mice acquired task contingencies in a similar way. Interestingly, most CA mice initially showed high action rate variability, that with time abruptly decreased, suggesting a shift to habitual performance. This shift was not evident in the WR mice. We also found signatures of automaticity in the licking patterns after animals transitioned to habit. The data suggest that habitual transitions during learning are abrupt and stable.

J20 Does the memory of a food source location can be modulated by the presence of a pheromone trail?

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Ant foragers must be able to locate a food source outside the nest and revisit it for exploitation. They are able to do this by following their memory and/or following a pheromone trail. In the present study we used workers of the Mediterranean ant *Crematogaster scutellaris* to evaluate how the presence of a pheromone trail affected the memory of a food source location. The behavioural assay was divided into 3 phases: A "Learning phase" where a single ant explored for 30 min a three compartment maze, an initial compartment where the ant was introduced that was connected to a right and to a left compartment. A sucrose reward was located either on the right or the left compartment. A pheromone trail, when present, was located form the entry point in the initial compartment to the food resource. Next, a "Trophallaxis phase" where the ant was allowed to enter in contact with a mark nestmate to do trophallaxis, and finally, a "Test phase", where the ants were reintroduced in the maze in the absence of food and pheromone trail and were allowed to explore. Our results indicate that *Crematogaster scutellaris* ants are able to learn and retain a food source location after, but were unaffected in their performance by the presence of a pheromone trail. This memory depended on the experience during the learning phase: the chance of choosing correctly increased with the number of visits to the reward compartment, and it was higher when the ant also ate during the visit. We decided to also examine the time required to enter the left or right compartment during the test phase. This latency to choose, in particular the compartment previously paired with food, could be interpreted as a proxy for memory. The latency to choose of the pheromone group was always lower than the one of the control group regardless of the choice made. Also, the latency to choose the previously food paired compartment, regardless of the treatment, was always lower than that of the ants who chose the previously empty compartment. In our setup, the chance to choose the correct compartment was not modulated by the presence of a pheromone trail during the learning phase: the memory was high and similar for both treatments. However the time to make this choice was always shorter for the pheromone group.

J21 Hierarchical architecture of dopaminergic circuits enables second-order conditioning in *Drosophila*

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Dopaminergic neurons with distinct projection patterns and physiological properties compose memory subsystems in a brain. However, it is poorly understood whether or how they interact during complex learning. Here, we identify a feedforward circuit formed between dopamine subsystems and show that it is essential for second-order conditioning, an ethologically important form of higher-order associative learning. The *Drosophila* mushroom body comprises a series of dopaminergic compartments, each of which exhibits distinct memory dynamics. We find that a slow and stable memory compartment can serve as an effective "teacher" by instructing other faster and transient memory compartments via a single key interneuron, which we identify by connectome analysis and neurotransmitter prediction. This excitatory interneuron acquires enhanced response to reward-predicting odor after first-order conditioning and, upon activation, evokes dopamine release in the "student" compartments. These hierarchical connections between dopamine subsystems explain distinct properties of first- and second-order memory long known by behavioral psychologists.

J22 Odd and even number categorisation by an insect and simple artificial neural network

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Categorisation of abstract concepts can be essential to how we understand and order complex information. The categorisation of numbers as odd or even is known as a parity task in human mathematical representations. Humans demonstrate accuracy, speed, language, and spatial relationship biases between categorising numbers as odd or even. For example, we are faster and more accurate when categorising numbers as even than odd. To date, there appears to be a complete absence of research exploring parity processing in non-human animals. We show that free-flying honeybees can visually acquire the capacity to differentiate between odd and even numerosities of 1 – 10 geometric elements and extrapolate this categorisation to the novel numerosities of 11 and 12. Contrary to humans, bees learnt to associate odd numbers with a reward more quickly than even numbers. We then constructed a simple artificial neural network consisting of five neurons that could reliably categorise odd and even numerosities up to 40 elements. While the simple neural network was not directly based on the biology of the honeybee brain, it was created to determine if a simple system could replicate the results we observed in honeybees. This study demonstrates that a task, previously only demonstrated by humans, is accessible to a brain with a comparatively small number of neurons. We discuss the possible mechanisms or learning processes allowing bees to perform this categorisation task, which range from numeric explanations, such as counting, to pairing elements and memorization of stimuli or patterns. We encourage further testing of parity categorisation in a wider variety of animals to determine its potential biological roots, evolutionary drivers, and if/how other non-human animals perform the task.

J23 Number neurons in the nidopallium of young domestic chicks

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Numerical competence is evolutionary important for animals to e.g. estimate a number of conspecifics in a group or a number of food items. Moreover, behavioural data from human infants and young domestic chicks suggest that number sense belongs to a core knowledge system and can be present already at birth. Previous studies revealed that the number sense relies on the activity of neurons that respond to abstract numerosities irrespective of their physical appearance. However, number neurons have been so far described only in the brain of adult animals. We aimed to test whether numerical abilities based on number neurons might be an inborn or spontaneously emerging property of the vertebrate brain. For doing so, we described neural responses to numerical stimuli in young and untrained domestic chicks. First, we habituated young chicks to a computer monitor, where numerical stimuli were presented. Then, we performed extracellular recordings in the caudolateral nidopallium (NCL) and explored neural responses to visual numerosities from 1 to 5. In the NCL of young, numerically naïve domestic chicks we found 53 neurons (11% of all units) that convey numerical information. Their firing rate significantly changed with numerosity, irrespective of the overall area/perimeter of stimuli. Response properties of number neurons in young chicks were similar to those described in adult animals: 1). they were tuned to specific numerosities and showed a decay in response to non-preferred numerosities; 2). the tuning curves of number neurons became wider with increased numerosity and more symmetrical when plotted on the non-linear scale (numerical magnitude effect). Our results suggest that numerosity perception based on number neurons is possibly an inborn feature of the vertebrate brain.

J24 Flexible recruitment during honeybee colony defence

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Honeybees defend their nest against large predators thanks to a collective effort to harass and sting the intruder. Typically the threat is detected by a few bees, which then need to quickly mobilize their nestmates into a defensive response. At the core of this recruitment is the sting alarm pheromone (SAP), a complex odour blend released when the bees' stinger is exposed. The SAP attracts and primes nearby workers for attack, thus

providing a seemingly simple communication channel during defensive events. However, when taking a closer look at this system we found that the behaviour of bees receiving this signal was dependent on a number of factors, both internal and external. We show that the likelihood for a bee to sting is linked to the concentration of alarm pheromone in a non-linear way, whereby responsiveness increases in the lower range of concentrations but decreases for very high concentrations. Such a dose-response curve could be important to prevent recruitment from getting out of control and depleting the colony of its workforce. We also found that the social context, in particular group size, plays a major role in regulating stinging behaviour and responsiveness to SAP. Finally, honeybees responded differentially to SAP as a function of their age: young (<10 days) and old (>30 days) bees had an increased likelihood to sting in the presence of SAP, while middle-aged (11-29 days) bees did not. Honeybees perform different tasks for the colony as they age, and this likely reflects this division of labour. Overall, our results suggest that recruitment by SAP is a finely-tuned mechanism. Our next challenge will be to understand the neurobiological processes underlying these modulations of SAP responsiveness.

J25 Genetic architecture of social and asocial learning in *Drosophila melanogaster*

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Social and asocial learning are required to cope with the complexity of the environment. However, whether they use a single (general-purpose) or distinct (special-purpose) cognitive mechanisms remains elusive. This study aims to address this question by studying the genetic architecture of social and asocial learning in *Drosophila melanogaster*. We firstly tested social and asocial learning abilities in 40 lines of the DGRP, a panel constituted of isogenic sequenced lines that together represent the genetic variation of a natural *Drosophila* population. We used aversive conditioning paradigms for oviposition sites. We obtained significant learning performance variation across the tested lines that showed no correlation between social and asocial learning. Secondly, we performed a GWAS between the genetic variants in the DGRP and each learning phenotype. We obtained two different sets of candidate genes for social and asocial learning, ranging from genes with unknown biological activity to genes already known to be related to learning. Thirdly, to functionally validate the role of each candidate gene on each learning phenotype, we used GAL4/RNAi-UAS lines, with a pan-neuronal knockdown. We found genes associated with specific learning types. Finally, we analysed the expression patterns of these genes in the *Drosophila* brain and found that some are expressed in the mushroom body, a brain region previously described to be involved in the associative learning process. Together, these results suggest, so far, the occurrence of a domain-specific genetic architecture for social learning in *Drosophila*.

J26 DnaJ/Hsp40 tunes long-term memory and functional amyloidogenesis

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Long-term memory (LTM) requires integration of both environmental and systemic cues and results in changes in synaptic protein composition and conformation. One such case is the amyloidogenic assembly of synaptic RNA-binding protein CPEB/Orb2, which drives formation and persistence of LTM. An important unanswered question is how environmental and systemic cues impinge on CPEB amyloid formation. Since chaperones are known to respond to environmental changes and regulate protein folding and assembly, it is possible that chaperones may regulate the formation and/or persistence of LTM by communicating changes in environmental or organismal states to synaptic protein state. As DnaJs comprise the largest and most diverse

family of chaperones, we have undertaken a screen involving overexpression and knockdown of the majority of fly DnaJs in the mushroom body, the seat of memory in insects. We have identified two previously uncharacterized DnaJ proteins, CG10375 and CG43322, whose overexpression improves both formation and retention of long-term memory. Intriguingly, we find evidence that CG10375 can potentiate memory even in the absence or attenuation of specific cues, while CG43322 relies in integration of several systemic and environment cues to potentiate memory. In a purified reconstituted system, CG10375 potentiates Orb2/CPEB amyloidogenic fiber formation. Taken together, these observations suggest an unexpected and specific role of DnaJ proteins in tuning long-term memory, and indicate a possible role for chaperones in the representation at the molecular level of experience or internal states in the nervous system. Our observations also suggest that there are chaperones in the brain that can promote amyloid formation and facilitate memory formation

J27 Feels like home: influence of size and chemosensory cues on scorpion shelter choice

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As scorpions belong to the rather understudied group of the Chelicerata, research on their behaviour is rare. But there is a pivotal task for these nocturnal animals: finding a save shelter for surviving daytime. Here, we address which role ambient conditions play in this context, namely size and scent. We conducted a two-choice shelter assay and tested 16 combinations of 2 shelter sizes (large, small) and 6 scents (neutral, female, male, cricket, rosemary oil, mineral oil). This was done by filming and analysing the behaviour of single scorpions during their entire night phase. Each morning the final decision was documented. We tested only females of two different species. *Euscorpius italicus* is found in moderate climate and *Mesobuthus eupeus* inhabits more arid regions. Comparing final hiding places, species from moderate and from arid habitat equally favoured the larger shelter in a neutral situation. The most striking differences concerned the smell of crickets (food) and rosemary oil (possibly aversive smell). *E. italicus* significantly favoured a small neutral shelter over a large shelter perfumed with rosemary oil. On the opposite *M. eupeus* significantly preferred a small cricket scented shelter over a large neutral shelter. These observed differences may be a result of the different habitats the scorpions live in. We also did an impairing experiment on the main sensory organs of the scorpions. We either impaired the pedipalps or the pectines. As a result, intact pectines seem to have slightly more influence on the final decision than intact pedipalps. Therefore, pectines may not only be important for contact chemosensation as already suggested by previous morphological studies, but possibly also for measurements of shelter sizes.

J28 Cephalopod brains revisited: smart snails or alien intelligence?

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Cuttlefish, squid and octopus are cephalopods famous for their large brain size and potential cognitive capability approaching that of small mammals. The brain layout and neural network complexity in all three was initially highlighted by Cajal and later JZ Young and colleagues. Recently, our work has sought to bring back to earth (sometimes literally) the thinking and hypotheses regarding the brains and behavioural boundaries of the cephalopods. Using new methods in high resolution magnetic resonance imaging (16.4Tesla MRI), golgi and other 'basic' anatomical techniques, combined with ecologically- and phylogenetically-informed comparisons, we have confirmed old and made many novel findings at the neuroanatomical level. We have confirmed that the deep sea vampire squid (*Vampyroteuthis*) possesses an ancestral-like brain configuration and its brain contains a mixture of features that support the idea of it as an intermediate between octopus and squid. Coastal cephalopods may be nocturnal or diurnal (e.g. octopus and cuttlefish) and mostly solitary (octopus) or partially social (e.g. cuttlefish and squid). These different ecologies and behavioural needs have driven differentiation of the optic lobe (vision), inferior frontal lobe (chemotactile) and vertical lobe (learning and memory). A comparison of the MRI-based cephalopod connectome along with neural trace ground-truthing shows that the diurnal octopus and cuttlefish have developed additional circuits in their vision and colouration systems related to their camouflage tactics in the bright and complex shallow water and reef habitats they dwell in. This new-technology update gives a firm base to a non-anthropomorphic interpretation of cephalopod comparative cognitive and behavioural abilities.

J29 Cuing bottom-up attention in bumblebees (*Bombus terrestris*)

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Attention allows animals to select relevant information from the constant sensory stimulation in their environment. In primates, attention can be willingly turned to specific stimuli (top-down attention) or captured by sudden or salient stimuli (bottom-up attention). Bottom-up attention has been shown to increase the perceived contrast of a target preceded by a cue. Here we investigated whether we can find similar processes in bumblebees in two different experiments. In a first experiment, bees were trained to drink a sugar reward at one of two artificial flowers situated on each side of green computer screen. The reward was indicated by a black circle displayed above the correct flower. Post training, bee attention was cued in a series of tests. In all tests with cuing conditions, a blue square cue was flashed either on the side of the target or the opposite side before the target appeared. A condition without a cue was used as a control. The target stimulus was presented after this cue with one of five different contrasts between a full contrast and zero contrast. We predicted that bees would detect the target at a lower contrast when the cue appeared on the same side compared to when the cue was on the other side or when it was not shown. In a second experiment, bees learned to choose a full contrast target instead of a distractor that could take various contrasts. During tests, a cue was presented as in the first experiment either on the side of the target, the side of the distractor or not displayed. Here, we predicted that the cue would hinder the bees' ability to discriminate the target from the distractor when it was on the side of the latter. We here present results for these two experiments and discuss the implications for attention-like processes in insects.

J30 Dynamic homeostatic plasticity within cerebellar circuitry

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The cerebellum has proved useful as a model system due to its uniform modular organization. The cerebellum of the mormyrid fish provides a special opportunity to directly determine how single PCs affect their target cells. Such an approach is nearly impossible in mammals. In this study of the mormyrid cerebellum, a Purkinje cell and its target cell were simultaneously patch-recorded in a slice preparation, as focal stimuli were used to activate the parallel fibers (PFs) or single climbing fiber (CF). Upon establishment of synaptic connection between two cells, three signals – PF responses in both cells and the synaptic inhibition in the postsynaptic cell – were recorded as controls, followed by the pairing PF and CF inputs to induce long-term potentiation (LTP) or depression (LTD) at parallel fiber synapses onto the presynaptic cell. Then, the three signals are recorded again and contrasted with the controls. Surprisingly, we found that after PF-LTP was induced in the presynaptic cell, the synaptic inhibition from the same cell to the postsynaptic cell was significantly attenuated; conversely, after PF-LTD was induced in the presynaptic cell, the synaptic inhibition from same cell to postsynaptic cell was significantly enhanced. These results indicate that synaptic plasticity at input synapses of the mormyrid Purkinje cell are not simply conveyed to the same cell's output synapses, rather that the strength of a PC's output synapses onto its target cells is up- and downregulated following LTD and LTP. We hypothesize that the runaway nature of Hebbian plasticity in a central neural network is buffered in an online fashion by changes generated by negative feedback at the same neuron's output synapse, a phenomenon which we have termed *dynamic homeostatic plasticity*.

METABOLISM, BIOLOGICAL RHYTHMS AND HOMEOSTASIS II

K7 Temperature-robust REM and SWS in *Laudakia vulgaris*

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During sleep our brain switches between two starkly different brain states - slow wave sleep (SWS) and rapid eye movement (REM) sleep. While this two-state sleep pattern is abundant across birds and mammals, its existence in other vertebrates is not universally accepted, its evolutionary emergence is unclear and it is undetermined whether it is a fundamental property of vertebrate brains or an adaptation specific to homeotherms. To address these questions, we conducted electrophysiological recordings in the Agamid lizard, *Laudakia vulgaris* during sleep. We found clear signatures of two-state sleep that resemble the mammalian and avian sleep patterns. These states switched periodically throughout the night with a cycle of ~90 seconds and were remarkably similar to the states previously reported in *Pogona*. Interestingly, in contrast to the high temperature sensitivity of mammalian states, state switches were robust to large variations in temperature (Q_{10} of 2.3). We also found that breathing rate and micro-movements were locked to the REM state as they are in mammals. Collectively, these findings suggest that two-state sleep is abundant across the agamid family, shares physiological similarity to mammalian sleep, and can be maintain in poikilothems, increasing the probability that it existed in the cold-blooded ancestor of amniotes.

K8 Exploring diel activity patterns, ecology, and genetics across hyperdiverse Lake Tanganyikan cichlids

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Many of the biological mechanisms that regulate the circadian phase, duration, and structure of diel activity are conserved across vertebrates. Given this extensive conservation, it is remarkable that activity patterns display widespread variation across species. However, the genetics and ecology underlying activity pattern diversity remain largely unknown. Cichlids represent the most extraordinary example of vertebrate explosive speciation — in the Rift Valley Lakes of Africa ~2000 species have evolved within the last 10 million years. In these lakes, cichlids have diversified to inhabit a vast array of ecological niches; diversifying in diet, body shape, mating behaviours, colouration, and habitat preferences. However, little is known about how their behavioural and diel activity patterns relate to their explosive radiation. We focused on a subset (>60) of the ~240 endemic Lake Tanganyikan cichlid species for which we have genomic, transcriptomic and ecological data. Using quantitative analysis of individual activity patterns over six days in the lab, we found that cichlids occupy all temporal activity niches, including diurnal, crepuscular and nocturnal patterns. Using GWAS we have identified genetic associations with temporal activity, which is remarkably labile between species and across the phylogenetic tree. While there were some enrichments of temporal activity with diet across species (perhaps reflecting food availability), temporal activity does not have exclusive associations with trophic level, indicating temporal niche differentiation. Our results reveal previously unrecognised temporal diversity in cichlids, which may have contributed to their extensive diversification and evolutionary success.

K9 Activity and energy: The effect of K-ATP channel activity on network output of the pyloric circuit in the *Cancer borealis* stomatogastric ganglion

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Prolonged changes in neuronal activity are triggers for homeostatic processes involved in maintaining activity levels around arbitrary 'set points'. ATP is intimately involved in maintaining a neuron's internal ionic environment and membrane hyperpolarization, especially in times of heightened activity. Fluctuations in levels of available ATP might serve as a readout of neuronal activity to balance its demand and supply. ATP-sensitive

K$^+$ channels have been implicated in regulating neuronal activity in response to changing glucose levels and hypoxia. Increased levels of ADP cause channel opening and strong membrane hyperpolarization and high levels of ATP cause depolarizations. We explore the role of K-ATP channels in regulating activity of the pyloric circuit of the crab stomatogastric ganglion (STG). The continuous rhythmic output of the network is maintained *in vitro* and has been studied for its remarkable homeostatic and adaptive properties to changing environmental conditions but the molecular underpinnings of these adaptations remain uncovered. We used the sulfonylureas tolbutamide and glibenclamide to block channel K-ATP activity channels and diazoxide to activate the channels. We found that pharmacological activation of K-ATP channels can result in a complete cessation of rhythmic activity in the intact circuit. Channels blockers, on the other hand, caused minor increases in oscillation frequency in the intact network. Removal of neuromodulatory inputs to STG neurons (decentralization) results in a dramatic reduction of network activity. Blocking K-ATP channels restored oscillatory activity in decentralized networks. Hence, we report the presence of a potent pathway in STG neurons for the modulation of their activity based on their intracellular ATP levels.

K10 Modulatory capacity correlates with dietary diversity in three species of decapod crustaceans

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The central nervous systems of decapod crustaceans contain numerous amines, amino acids, and peptides that modulate the central pattern generating (CPG) circuits found within its stomatogastric nervous system (STNS) and cardiac ganglion (CG); over 30 modulators have been identified in one species of crab (*C. borealis*). CPGs in the STNS are responsible for internally chewing and filtering food into the midgut, while the CG drives the heartbeat. Neuromodulation allows these CPGs to flexibly produce a variety of output patterns; however, the variety of modulators exceeds the variety of induced output, leading us to ask why so many modulators are present. Although modulatory capacity, a measure of the extent to which a neuronal circuit can be modulated, may be solely an inherited trait, we hypothesize a functional correlation, with decapods that need more behavioral flexibility utilizing a broader range of modulators than those with more stereotyped behaviors. We thus predicted that decapods with broad diets would have a larger modulatory capacity in the STNS, but not the CG, than those with limited diets. To test this, we compared responses of the STNS and CG CPGs in three species of majoid crabs (*P. producta*, *L. emarginata*, and *C. opilio*) to the same neuromodulators. *L. emarginata* and *C. opilio* both have broad diets, while *P. producta* primarily eats kelp. With one exception, each species' CG exhibited similar responses to all modulators. The STNS of crabs with broader diets responded similarly to all modulators, whereas the *P. producta* STNS showed limited responses to the same set of neuromodulators. CG responses did not follow this trend, as all crabs showed varied CG response to modulation.

K11 Temperature responses of stomatogastric neurons in the brush-clawed shore crab, *Hemigrapsus takanoi*

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The intertidal brush-clawed shore crab, *Hemigrapsus takanoi*, is native to the temperate waters of Japan. It has recently seen a dramatic invasive expansion into colder European waters, but the reasons for this expansion remain unclear. Here, we investigate the temperature range at which the nervous system of *H. takanoi* remains functional as a possible aspects of this species' invasive success. Since the body temperature of intertidal invertebrates closely follows the rapidly changing temperatures in this habitat, neurons are predicted to function over a wide temperature range to ensure survival and facilitate the species' range expansion. We analyzed *in vitro* temperature responses of the pyloric central pattern generating neurons in the stomatogastric

ganglion. The pyloric rhythm serves a vital function in digestion. Like in other decapod species, it was continuously active with a triphasic pattern and well-maintained phase relationships (N>10). However, in contrast to other crabs, pyloric rhythm frequency increased only moderately with temperature, reaching a maximum of <1 Hz below 20°C. Unexpectedly, pyloric phase relationships changed, with warmer temperatures increasing the duty cycle of the pyloric PY neurons. The pyloric rhythm was very cold-resistant. First action potentials failed at 2.7±0.4°C (N=8). Rhythmic activity stopped at 0.8±0.3°C (N=7). At high temperature, individual action potentials or bursts failed at 30.5±0.9°C (N=10). The rhythm crashed at 31.7±0.9°C (N=10) when neurons started to fire in unpredictable ways. A return to room temperature restored rhythmic activity in all cases. Thus, *H. takanoi* neurons remain functional over a range of almost 30°C. We are currently testing the effects of long-term changes in habitat temperature on neuronal activity.

K12 Network responses to changes in extracellular saline concentration in the lobster *Homarus americanus*

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Climate change has resulted in altered patterns of rainfall globally. Consequently, sea water is both increasing and decreasing in salinity as a function of decreased and increased rainfall patterns respectively. This includes changes in both surface and water column salinity. This is particularly concerning for organisms with open or semi-open circulatory systems as they exchange seawater with their internal environment. This is potentially problematic for the nervous systems of these organisms. Neuronal physiology is determined by the expression of ion channel conductances, the ionic gradients established across a neuron's membrane, and the relative equilibrium potentials of those ion species. Intracellular ionic concentrations are generated by both active (pumps) and passive (leak channels) transport. The equilibrium of a given ion species results from its membrane permeability, charge, and the presence of impermeable, intracellular anionic molecules (e.g. DNA and proteins). Consequently, changes in the extracellular concentrations of all permeable ion species will affect neuronal physiology. Here we investigate how altering the concentrations of all ion species in the extracellular solution affects neural circuit function. Using the stomatogastric nervous system and cardiac nervous system of the American lobster (H. americanus), we demonstrate that these nervous systems, which control movements of the foregut and heart respectively, are each able to maintain physiological function when challenged with both increased and decreased saline solutions applied to the extracellular environment (to a certain extent). Interestingly, each nervous system is more tolerant to decreased saline solutions compared to those with increased salinity.

METHODS AND EDUCATION

L1 A method for stereotaxic brain surgery without a brain atlas nor a standard stereotaxic frame

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Acquiring experimental data in systems neuroscience often relies on stereotaxic surgery for precise positioning of electrodes and probes. While stereotaxic surgery can be highly precise, its major drawback is that it can only be performed in species for which a brain atlas and a standard stereotaxic holding frame have been developed. Unfortunately, this leaves only a hand-full of species, mostly standard animal models, such as mouse, rat, zebra-finch etc. To overcome this obstacle we developed an alternative technique for precise brain surgery. To apply our technique, a high contrast MRI scan of one specimen and an access to a micro CT scanner are required. The first stage is a surgery to attach miniature metal markers to the skull surface. The second stage is a CT scan of the animal's skull, under anesthesia. The third stage is done off-line: the MRI and CT 3D images are co-registered using a standard image processing software (Fiji, NIH), followed by marking the desired brain targets in the merged MRI images. The final stage is the surgery for which the head is fixed in any convenient orientation. The

probe, to be inserted, is attached to an XYZ positioning system and its 3D position and orientation is tracked with a stereo video system. We developed a software that uses the metal markers on the skull as fiducial markers to transform the coordinate system of the CT/MRI 3D model of the brain to the coordinate system of the positioning system, and in turn, to guide the surgeon how to move the probe. This technique allows executing insertion tracks connecting any two points in the brain. We used this method for positioning of Neuropixels probes in owls and quails. The technique enables efficient studies in non-standard and new animal models.

L2 Ants 3D pose tracking during grasping behaviour

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Despite a rather limited number of neurons, insects display a wide range of complex behaviours and fine motor skills. One particularly fascinating example is the ability of ants to manipulate a variety of items of different shapes, textures, and weights ; a versatility that is central to the polyethism found across ant species (with specific needs in nest-building, brood care, foraging, etc). It is thought that most of the sensory information acquired prior to a grasp attempt originates from tactile active sensing with the antennae, but details remain unclear and we know very little about the underlying 'cognitive' and neurobiological aspects. It seems implausible that ants would be able to compute a full 3-dimensional representation of the objects or the action-space ; still, their efficiency at grasping various items, especially in clutter, often outclasses even the best robotic and computational approaches. In order to understand the sensory mechanisms and motor functions that enable effective selection, positioning and (re)adjustment of a grasp, we develop a high-resolution motion capture arena to accurately record and track ants interacting with different things. The arena consists of 5 synchronised high-speed cameras arranged orthogonally around and above the ant, associated with a deep-learning-based pipeline to recover the full 6-DOF of the head, mandibles and antennae. We hope to use this setup in an extensive range of experiments with different items and mechanical constraints. Ultimately, we hope to apply some of the efficient solutions proposed by the ant brain to a real-life grasping task of unknown objects, all the while avoiding the need to develop complex new effectors, given the existing similarity between ants' mandibles and standard robotic grippers.

L3 Crescent Loom: a flexible neurophysiology online simulation for teaching neuroethology

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Simulations are useful tools for teaching principles of neurophysiology, circuit-thinking, and hypothesis testing in contexts where wet-lab activities may be inaccessible. The Crescent Loom Connectome Explorer is an *in silico* tool in which players can relate the structure and function of central pattern generators to animal behavior. Students are given a circuit with obscured connectivity and the tools to do experiments (e.g. blocking neurotransmitters, stimulation) while recording from neurons and observing the animal's behavior in order to generate a prospective connectivity map. We have used the flexibility of this tool to develop and implement activities to teach a range of concepts in neuroethology for introductory through advanced undergraduate student audiences (4 courses at 2 institutions; >200 students). Activities illustrate concepts such as models of CPG organization, the role of intrinsic properties in CPG function, and the role of comparative approaches in understanding links between physiology and behavior. We will discuss assessment results of selected classroom activities and present choices for implementing the Crescent Loom Connectome Explorer given particular learning goals and contexts.

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