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CONFERENCE ABSTRACTS

International Society for Neuroethology

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OPEN BOOKMARKS

Oral Abstracts

JCPA Presidential Symposium

[PS2] As the craw flies and the beetle rolls: Straight-line orientation from behaviour to neurons

Professor Marie Dacke[1]
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The seemingly simple act of walking in a straight line involves a complex interplay of various sensory modalities, the motor system, and cognition. This is obvious to anyone who have ever found themselves lost in the desert at night, or in a forest when the sun is high in the sky. A dung beetle released in the same unchartered territory does not move in circles, but holds a chosen bearing until it encounters a suitable spot to bury its ball of dung. The key to the beetle's success lies in their ability to detect and orient via a large repertoire of celestial compass cues, from the bright sun to the weak intensity differences of light provided by the Milky Way. A beetle's drive to adhere to its set course is so strong that it sticks to it regardless of the costs; over stones, through bushes and grass or in an experimental arena. However, if a beetle is forced to make a new ball, the bearing information is reset in its brain and a new course is set. This unique and robust orientation behaviour, in combination with an accessible brain, make the dung beetle an ideal model system for understanding the fundamental visual and neural processes underlying straight-line orientation. The presentation provides an overview of recent behavioural, anatomical and physiological results concerning how an insect brain is designed to facilitate straight-line orientation.

[PS3] The widespread and long-term evolutionary consequences of human behaviour

Dr George Perry[1]

The subsistence and habitat-modifying behaviors that typify how humans interact with our surrounding environments can have broad and temporally profound effects on natural ecosystems. In addition to ecosystem health and wildlife extinction risk, human behavior also influences the evolutionary biology of non-human species. Extensive human-driven behavioral and morphological phenotype evolution has been thoroughly documented for domesticated plants and animals, but these processes are in fact much more widespread, extending to non-domesticated species. Moreover, archaeological records suggest that similar processes extend considerably into prehistory, perhaps to 50,000 years before present or earlier. I will discuss the various processes by which human behavior can and has driven non-human evolutionary biology, including with examples from integrated phenotypic, evolutionary genomic, and ancient DNA research studies that are ongoing in my laboratory, and how these studies are also helping to reconstruct past human behavior by proxy.

[PS4] Senses for three-dimensional orientation in C. elegans

Professor Jon Pierce[1]
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C. elegans was chosen as a model by Sydney Brenner primarily for the promise of understanding whole-animal development and behavior at the level of identifiable cells and entire neuronal circuitry. At the time, Brenner, among others, lamented that the worm appeared relatively disadvantageous for behavioral complexity. By comparison, Drosophila boasts impressive behaviors including flight and vision. However, the complexity of worm behavior may be underestimated. By imagining how worms behave in their natural 3D environment of rotting vegetation rather than on the 2D surface of agar-filled Petri plates, we may discover that the worm is capable of complex behaviors. While burrowing vertically through compost, the worm would encounter an array of cues including gaseous, moisture, and geomagnetic that could guide it to a favored microenvironment. Adopting this mind frame, our lab has recently demonstrated that C. elegans performs a variety of sophisticated behaviors with implications for other animals. The worm orients to subtle humidity gradients, burrows using genes important for human muscular dystrophy, and directs burrowing by sensing the Earth's magnetic field. By adopting the imagination of classical neuroethologists, C. elegans researchers will likely discover a rich repertoire of behaviors.

[PS5] Molecular insights into the evolution of mosquito preference for human odor

Assistant Professor Carolyn (Lindy) MCBride[1]
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Researchers investigating the outbreak of an unknown illness along the coast of East Africa in 1952 discovered homes inhabited by a ‘domestic’ form of the mosquito Aedes aegypti. An ancestral ‘forest’ form of the same species was later found breeding in forests, just hundreds of meters away. Although closely related and fully interfertile in the laboratory, the two forms remained distinct in the wild and showed striking divergence in behavior: Domestic females specialized on biting humans, readily entering homes, flying toward human odor, and laying their eggs in water-storage containers indoors. Forest females avoided homes, preferred the odor of non-human animals, and laid their eggs in tree holes outdoors. These behavioral differences translated into marked divergence in capacity to spread human diseases, such as Chikungunya, the unknown illness from 1952, yellow fever, prevalent in Africa and South America since the 16th century, and dengue fever, currently causing sickness in over 300 million people around the world each year. This story illustrates how marked and complex behavioral differences can evolve between closely related populations in nature with profound implications for human health. In my laboratory, we are taking advantage of such evolution to uncover the genetic and neural basis of behaviors that adapt mosquitoes to human hosts. Using the mosquitoes described above as a model, we are working to identify the genetic changes that underlie the innate preference of domestic females for human odor and determine how these changes alter the activity and structure of olfactory circuits to generate distinct behavioral responses.

Plenary Lecture 1

[PL1] Insights into the evolution of parental behavior from poisonous amphibians

Professor Lauren O'connell[1]
[1]Stanford University, Stanford, United States

Parental care is a key evolutionary innovation that facilitates the exploitation of novel habitats, influences fitness and survival of parents and offspring, and serves as an evolutionary precursor to the emergence of social behavior. Various parental care strategies have evolved in multiple taxa, yet the underlying mechanisms promoting the evolution of these behavioral phenotypes are poorly understood. Poison frogs show remarkable variation in parental care strategies including male- and female-uniparental care and biparental care. Importantly, paternal and maternal care occur with and without pair bonding in this clade, allowing disentanglement of parental care behaviors from pair-bonding. Parental care in poison frogs involves defense and hydration of embryos during development, and transportation of tadpoles by piggyback to pools of water. In species in which females care for offspring, mothers nourish growing tadpoles with trophic eggs until metamorphosis is complete. I will first discuss tadpole transport behavior that involves comparative work across three closely related species and has given us insights into general themes on parental care neural circuits across sexes and species. Specifically, we have found that the hypothalamus and hippocampus are critical in promoting parental care in frogs and have identified the neuromodulators that promote these behaviors in amphibians and other vertebrates. Second, I will zoom out to look at the convergent evolution of maternal care in two independent evolutionary origins of poison frogs in South America and Africa. In our focal species that diverged 150 million years ago, mothers provide trophic, unfertilized eggs to their developing tadpoles. Both species lace these eggs with neurotoxins, providing insight into how these energetically costly behaviors are maintained. Although we find convergence at the level of behavior, we have found that the molecular mechanisms underlying these behaviors are different. Finally, we will explore the parent-offspring interactions that facilitate this egg-feeding behavior by examining the neural basis of begging behavior in tadpoles. Tadpole begging behavior is an honest indicator of need where tadpoles must make life-or-death decisions about whether or not to beg in response to a visitor to their bromeliad, who may be their mother or a predator. Moreover, the tadpoles must tune these energetically costly begging displays with overall nutritional state. Overall this body of work is providing insight into the evolution of parent-offspring interactions and highlights the importance of comparative work based both in the lab and in the field in contributing to our understanding of behavioral evolution.

Young Investigator Award Symposium

[YI1] Spatial summation in hawkmoth lamina monopolar cells.

Dr Anna Stöckl[1,2], Prof. David O'Carroll[1], Prof. Eric Warrant[1]
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Many nocturnal animals rely on vision as their primary sense. The challenging conditions at night – weak signals and several sources of noise – are met by adaptations in their eyes and retina. Some nocturnal insects acquire additional sensitivity by summing visual signals in space and time in their brain (Stöckl et al. (2016) Curr Biol. 26:821-26). This boosts the correlated signals while averaging out uncorrelated noise, yet at the expense of spatial and temporal resolution. However, the neurons responsible for this processing remain unknown. Lamina monopolar cells (LMCs) – the primary relay neurons of the lamina, the insect brain’s most peripheral visual processing area – have been hypothesized to spatially pool information via their lateral dendrites. Here, we tested the hypothesis that lamina monopolar cells perform spatial summation for the first time using physiological methods in the hawkmoth Macroglossum stellatarum. We recorded LMCs intracellularly and morphologically identified them according to a previous classification (Stöckl et al. (2016) J Comp Neurol. 524:160–175). We characterised the spatial responses of LMCs at a range of light intensities and showed that: (1) LMCs were 100 times more sensitive than photoreceptors, but their spatial resolution decreased with light intensity. (2) The responses of LMCs matched previously quantified spatial summation estimates in the motion system of M.stellatarum (Stöckl et al. (2017) Proc R Soc Lond B. 284:20170880). (3) The spatial receptive fields of the LMCs matched their anatomically quantified dendritic extents. Together, these findings provide the first physiological evidence for the decades-old hypothesis that spatial summation is performed by lamina monopolar cells in nocturnal insects.

[YI2] Tadpole fight club: Neural mechanisms of conspecific juvenile aggression in poison frogs

Dr Eva K Fischer[1], Randi Petrillo[2], Gwen Ellis[2], Kate M Lagerstrom[1], Dr Lauren A. O'Connell[1]
[1]Stanford University, Stanford, United States, [2]Harvard University, Cambridge, United States

Resource competition is a major driver of aggressive interactions among conspecifics, both at acute and evolutionary timescales. Aggressive interactions among siblings competing for access to parental care is well documented; however, mechanisms mediating juvenile aggression are poorly understood. In poison frogs, increased parental care is associated with decreased water volume of tadpole deposition sites and increased resource competition. This resource competition has led to the evolution and maintenance of aggression in poison frog tadpoles. Indeed, many poison frog tadpoles will attack, kill, and cannibalize other tadpoles. While this link between resource limitation and aggression is documented in a handful of species, underlying neural and physiological mechanisms are completely unexplored. To address this question, we examined the neural basis of conspecific aggression in Dendrobates tinctorius poison frog tadpoles. We compared patterns of generalized neural activity as well as specific candidate molecules across tadpoles that won aggressive interactions, lost aggressive interactions, or did not engage in a fight. We found that distinct patterns of neural activity predicted the extent of aggression individuals displayed. Furthermore, increased activity in vasotocin (the non-mammalian homologue of the nonapeptide arginine vasopressin) neurons specifically was associated with increased aggression. Our findings shed light on neural mechanisms mediating aggression in poison frog tadpoles. Given widespread functional conservation of the neural mechanisms underlying social behavior, we suggest these mechanisms may contribute to juvenile aggression across vertebrates.

[YI3] Songbirds can associate arbitrary visual cues with learned song modifications

Dr Lena Veit[1], Mr Lucas Tian[1], Mr Christian Hernandez[2], Dr Michael Brainard[1]
[1]University of California San Francisco, San Francisco, USA, [2]University of New Orleans, New Orleans, USA

Bengalese finch (Lonchura striata) song is a complex learned motor skill with variable sequencing. In addition to initial song learning during development, adult Bengalese finches can be trained to modify the pitch or sequencing of individual song elements through reinforcement learning. Here, we tested whether adult birds additionally have the capacity to flexibly switch between learned changes to their song if they are provided with contextual cues indicating that different song modifications are adaptive in different contexts. In a pitch learning experiment, we paired opposite directions of pitch reinforcement for the same song syllable with different colors of cage illumination, e.g., reinforcing upward pitch shifts in orange light and downward pitch shifts in green light. In a sequence learning experiment, different light colors were paired with aversive feedback delivered to either of two alternate syllable sequences at a point in the song with naturally variable syllable sequencing. After training birds on these protocols, light switches elicited immediate adaptive changes to the song that minimized the aversive feedback in each context. These changes were apparent in the first song bout after light switches, as well as in probe contexts without reinforcing feedback. These results indicate that Bengalese finches can learn to associate arbitrary contextual cues with specific changes to both the pitch of individual syllables and to syllable sequencing at branch points. This suggests that the song system can store opposing biases at different levels of song control and rapidly reconfigure motor output in response to arbitrary learned visual cues.

[YI4] Mechanosensory and visual integration in the fly central complex

Dr Nicholas Kathman[1], Dr Jessica Fox[1]
[1]Case Western Reserve University, Cleveland, United States

The reduced hindwings of flies, known as halteres, are specialized mechanosensory organs that detect inertial forces associated with body rotation during flight. Previous studies have shown that primary afferents of the haltere encode its oscillation frequency linearly over a wide bandwidth and with precise phase dependent spiking. It is not currently known whether information from haltere primary afferent neurons is sent to higher brain centers, or whether precise spike timing is useful beyond the peripheral circuits that drive wing movements. We show that in cells in the central brain, the timing and rates of neural spiking can be modulated by sensory input from the haltere. We examined responses of central complex (CX) cells in a flesh fly to a range of externally imposed haltere oscillation frequencies and visual motion speeds. Haltere-responsive units fell into multiple response classes, including those with firing rates linearly related to the haltere frequency and others with responses independent of frequency. Most of these units also respond to visual motion, often in a directionally selective manner. When both stimuli were presented simultaneously, these visual responses increased in magnitude but maintained their direction and speed sensitivities. Although haltere inputs have largely been studied in the context of rapid flight control, we have found haltere sensory information in a brain region known to be involved in slower, higher-order behaviors, such as navigation. This data, along with recordings from tethered walking animals, may indicate a role in behaviors that take place over a longer timescale.

S1: Challenging the notions of pain, problem-solving and cognitive ability across taxa

[S1-1] Comparative Cognition and welfare – a fishy perspective

Dr Paul Hardy-Smith[1], Culum Brown[2]
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Fishes have long been maligned as mindless automatons, but research over the last 20 years has clearly demonstrated that their “3 second memory” status is ill deserved. On the contrary, comparative cognition has tended to focus more on the similarities between fishes and the rest of the vertebrates rather than the differences, so much so that fish are rapidly replacing lab rats as the go to experimental taxa. Comparative cognition as a field has shifted to examine the ecological drivers of cognition rather focussing on phylogenetic closeness to humans as an indicator of intelligence. With more than half the world’s vertebrate taxa, fish are an extremely valuable model group for research in this context. Here we summarise some of the work examining cognition in fishes and why television advertisements which play on the 3 second memory of fish may be based on false facts.

[S1-2] Third-party knowledge and ‘politics’ in ravens

Professor Thomas Bugnyar[1]
[1]University Vienna, Vienna, Austria

A core feature of social intelligence is the understanding of third-party relations. While some primates have been experimentally demonstrated to categorize the relationships of group members according to kin and dominance status, little is known about this capacity and flexibility of its use from other animals. Adopting the playback design used in primates, we here tested captive ravens for their ability to differentiate between simulated dominance interactions that either confirmed or violated the current rank hierarchy of members in their own social group and, critically, also of ravens in a neighboring group with whom they never had any physical interactions. Birds responded with increased stress and activity patterns to playbacks that were incongruent to the dominance hierarchy of their own group and a decrease in vocalization and attention patterns when the incongruent playbacks concerned the neighbouring group. These findings indicate that ravens are capable of deducing third-party relations not only via physical interaction with some of the subjects (own group) but also by mere observation (neighboring group). We discuss these skills in respect to challenges imposed by the high degrees of fission-fusion dynamics shown by ravens under field conditions and recent observations on wild ravens selectively interfering in agonistic and affiliative interactions of conspecifics.

[S1-3] Is mental template matching a cultural transmission mechanism in New Caledonian crows?

Dr Alex Taylor
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The human capacity to cumulatively evolve technologies and traditions is thought to depend on a unique suite of cognitive abilities, including teaching, language and imitation. Arguably, non-human animals, lacking this suite of abilities, cannot possess cumulative cultures. However, it remains possible that less elaborate cognitive mechanisms might offer some alternative routes towards cumulative cultural evolution in other species. Tool-making New Caledonian crows manufacture a variety of hook tools in the wild and may possess a material culture that has incorporated incremental changes over time. These crows do not appear to imitate, teach or possess language; however, their varied tool designs could be culturally transmitted through a process of mental template matching. That is, individuals may use or observe the products of tool manufacture, form a mental template of a particular tool design, and then reproduce this in their own manufacture – a process analogous to birdsong learning. I will discuss recent evidence from our lab suggesting that New Caledonian crows may have the cognitive machinery for mental template matching, a mechanism that could support the cumulative evolution of material cultures.

[S1-4] Who needs a brain? Problem solving and decision making by a giant amoeba

Dr Tanya Latty[1]
[1]University Of Sydney, University Of Sydney, Australia

All living organisms need to process information in one form or another- this basic ability allows them to find and exploit the resources necessary for life. The vast majority of research into problem solving and decision making focuses on organisms with brains, despite the fact that the majority of life forms on the planet – greater than 97%, - are brainless. The 'brainless majority' includes large and ecologically significant groups such as plants, fungi and microorganisms. Brainless organisms face many of the same challenges as their brained counterparts but must somehow solve them in the absence of a dedicated information processing organ. In this talk, I will discuss decision making and problem-solving in a giant amoeba: the slime mould, Physarum polycephalum. Despite being unicellular (and therefore brainless), Physarum polycephalum can make trade-offs between risk and food quality, adjust its search strategy depending on environmental quality, balance its macronutrient intake by selectively choosing complementary food items, solve mazes, and may even display forms of memory. Slime moulds are also subject to many of the same cognitive peculiarities as are brained organisms, exhibiting speed-accuracy trade-offs when making decisions under stress and human-like 'irrationality' when confronted with irrelevant decoy options. How do slime mould process information and what does this mean for the way we think about behaviour and cognition? What can slime moulds teach us about the evolutionary origins of decision making, problem solving and cognition? I will finish the presentation by discussing the need to better understand decision-making in the 'brainless majority'.

S2: From perception to action: Roles of auditory input in shaping vocal communication and social behaviours

[S2-1] Duets in the wild: Interindividually coordinated premotor neural activity enables cooperative behavior in songbirds

Dr. Susanne Hoffmann[1], Lisa Trost[1], Dr. Cornelia Voigt[2], Dr. Stefan Leitner[1], Alena Lemazina[1,3], Hannes Sagunsky[1], Markus Abels[1], Sandra Kollmansperger[1,3], Dr. Andries Ter Maat[1], Dr. Manfred Gahr[1]
[1]Max-Planck-Institute for Ornithology, Seewiesen, Germany, [2]University of Pretoria, Pretoria, South Africa, [3]Ludwig-Maximilians-Universität München, Munich, Germany

Many organisms are able to coordinate rhythmic motor actions with those of a partner to generate cooperative social behaviors such as duet singing. Recent neuroimaging studies in human subjects showed that the interindividual coordination of motor behavior during rhythmic social interactions is associated with a synchronization of brain waves between interaction partners. On the cellular level, the precise neural mechanisms that enable rhythmic interindividual coordination of motor actions are still unknown. Here we used duetting songbirds as a model system to investigate how premotor neurons enable the interindividual coordination of vocal production during duet singing. We exploited a newly developed radio-telemetric method to first record extracellular premotor activity and individual vocalizations simultaneously in free-living pairs of duetting birds. We found that the onset of a partner's contribution to the duet triggered a change in rhythm in the periodic neural discharges of the bird initiating the duet. The resulting phase-locked pattern of premotor neural activity between both birds in turn caused the precisely coordinated production of alternating duet elements that is characteristic for this species' song. Our results indicate that rhythmic cooperative behavior requires the precise interindividual coordination of premotor activity, which can be achieved by integration of sensory feedback originating from the respective interaction partner.

[S2-2] Time varying information about the meaning of vocalizations in the avian auditory cortex

Dr Julie Elie[1], Prof Frederic Theunissen[1,2]
[1]Helen Wills Neuroscience Institute, University Of California Berkeley, Berkeley, United States, [2]Department of Psychology, University of California Berkeley, Berkeley, United States

Understanding what and when information is encoded in neural responses is a central question in neuroscience. In sensory systems, it is assumed that neurons encode information about the stimulus, but what aspect of the stimulus is encoded and when does it happen can be difficult to identify. We developed a new method for calculating time-varying mutual information between the stimulus and the response and applied it to single unit spike trains in high-level auditory cortical areas. This time-varying information is calculated as the non-redundant cumulative mutual-information starting at the beginning of the stimulus presentation. It is obtained by modeling the stimulus dependent spiking statistic as an inhomogenous Poisson process and calculating the joint distributions of stimulus and responses by Monte-Carlo sampling. We applied this calculation to the responses of single auditory neurons that we recorded in the different regions of the avian auditory cortex: thalamo-recipient primary auditory cortex and secondary auditory areas of zebra finches. The stimuli consisted of a large number of biologically relevant vocalizations: multiple renditions from multiple birds of the entire repertoire of zebra finch communication calls. These calls are classified along 9 semantic categories based on their acoustics, behavioral context and responses. Comparing time-varying spike rates and time-varying information, we first demonstrate that an increase in spike rate is not synonymous to an increase in information. Second, we are able to parse out the sound stimulus information from the semantic category information, revealing that information about the semantic category has a longer latency. Finally, the time-course of information across neurons and cortical brain regions can also be analyzed to obtain a picture of the information flow. Taken together, these results demonstrate how information flow about relevant biological features (here the meaning of communication calls) can be revealed using the spatial and temporal resolution of electrophysiological responses.

[S2-3] Mechanisms for the control of duet singing in plain-tailed wrens

Professor Eric Fortune
[1]New Jersey Institute of Technology, New Jersy, United States

Plain-tailed wrens (Pheugopedius euophrys) are a member of a group of neotropical songbirds that produce complex duet songs. These songs are used by pairs of wrens in territorial defense and are believed to have additional roles related to reproductive behaviors. Our primary area of interest is the behavioral and brain mechanisms that the birds use to coordinate duet performances. The birds use acoustic cues from their partner, known as heterogenous feedback, to control the temporal and spectral features of song syllables. For example, when duetting wrens initiate singing with large distances between the birds, from over 2 meters to at least 14 meters, birds will fly towards each other to reduce acoustic delays, thereby improving the temporal coordination of the duet performance. We have also observed that birds will match the acoustic parameters of duet playbacks. The function of this matching is not well understood, but may reflect the learning process in which females and males learn sex-specific categories of syllables. We also investigated the neurophysiological mechanisms by which heterogenous feedback affects duet coordination by implanting tetrodes into HVC (a sensorimotor telencephalic area involved in song control) while the birds produced duets. We found sex differences in singing- and hearing-related activity that appear to reflect differences in the roles of females and males in the coordination of duet performances. Heterogenous feedback inhibits activity in HVC of awake females but not males. Similarly, singing context affects the magnitude of premotor activity in females but not males. When birds were anesthetized with urethane and their duet songs played back, HVC neurons in males showed a dramatic shift in responsiveness towards female syllables whereas female syllables responded equally well to both male and female syllables. These data may support the hypothesis that females provide leading cues for the duet.

[S2-4] Neural Mechanisms of Song Evaluation and Mate Choice in Female Songbirds

Dr Jonathan Prather[1], Jeffery Dunning[1], Koedi Lawley[1]
[1]University Of Wyoming, Laramie, United States

Female songbirds are highly skilled in perceiving the detailed features of male song and using the information contained in that signal to choose their mate from among many suitors. In that decision-making process, females evaluate the quality of a sensory experience and use that information to direct specific motor responses. We are very interested in understanding the circuits and the patterns of cellular activity through which that decision-making process occurs. In our studies of songbirds, a key experimental advantage is that song is so effective in influencing female preference that females will perform behavioral indicators of mate choice (i.e. copulation solicitation displays and calls) in response to song even if no male is physically present. Studies of female responses to song have implicated several auditory cortical regions and brainstem motoneuron sites in the expression of mate preferences. Here, I will discuss our recent and ongoing studies of Bengalese finches (Lonchura striata domestica) to develop a reliable behavioral test of female mate preference, to identify the features of song that make it attractive to females, and to discern the connectivity and respective contributions of the associated brain sites and the synaptic targets through which they influence motor output.

S3: Neuroethology of distributed visual systems: How do many-eyed animals perceive the world?

[S3-1] Neuroethology of the distributed visual systems of bivalves, chitons, and fan worms

Dr Michael Bok[1], **Dr Daniel Speiser[2]**
[1]University of Bristol, School of Biological Sciences, , United Kingdom, [2]University of South Carolina, Department of Biological Sciences, , United States

Most research on visual processing has been conducted on animals that have a pair of eyes located on their head. Generally, information gathered separately by the right and left eyes is integrated so that a single reconstruction of the visual environment is formed. Such integration is necessary if an animal is to accurately gauge the number and relative positions of objects in its environment. Paired cephalic eyes are not the only eyes found in the natural world, however, and we may have much to learn from visual systems that deviate from this familiar arrangement by incorporating dozens or hundreds of separate image-forming structures. Currently, we are studying the distributed visual systems of scallops, chitons, and fan worms to learn if these invertebrates integrate the images gathered by their many separate eyes. As initial steps in this process, we are 1) studying how neuroanatomy differs between scallops, chitons, and fan worms that have eyes and closely-related species that lack eyes, and 2) using the micro-injection of lipophilic dyes to trace the paths of the optic nerves exiting these eyes. Here, we are particularly interested in the spatiotopic mapping of eyes to the visual processing centers of these animals. We are also using comparative approaches to study how and why dispersed visual systems evolve. Here, our questions include: What were the ancestral sources of the separate components of these visual systems? Do dispersed visual systems tend to be associated with certain ecological functions? And why do dispersed visual systems appear to be associated with centralized processing in some taxa but not others?

[S3-2] From compound eyes to arrays of single eyes in insects

Professor Elke K Buschbeck[1]
[1]University Of Cincinnati, Cincinnati, United States

Arthropods are well-known for their diversity of eye types. For example, adult insects are typically characterized by compound eyes, whereas their larval forms often carry several single eyes that are dispersed around their heads. Two insects are particularly noteworthy. The twisted-wing insect Xenos peckii is characterized by an eye that looks much like a compound eye, but is however composed of about 50 units, each of which functions as a small image forming eye. Information from all these eyes then is pooled into a single visual system. A second example is that of the larvae of Thermonectus diving beetles, which have 6 image-forming eyes on each side of their head. These eyes most likely evolved from a single compound-eye like ancestor, which left a distinct signature in their development. Just like for compound eyes, Thermonectus larval eyes develop from a common epithelium of precursor cells. In both cases first photoreceptors, then support cells and then the lenses develop. In Thermonectus larvae the developing stemmata then separate and migrate into different positions, leading to a distributed visual system. A molecular analysis reveals that deeply conserved eye development genes underlie both systems. Relatively minor changes in eye development hence could have lead to a distributed visual system in Thermonectus. In these diving beetles stemmata have separated sufficiently for photoreceptors to project into separate neuropils, but so far it remains unclear if the visual pathway converges at later processing stages.

[S3-3] Star-gazing on the reef: can brittle stars 'see'?

Dr Lauren Sumner-Rooney[1], Dr Esther Ullrich-Lüter[2], Dr Imran Rahman[1], Mr John Kirwan[3], Dr Julia Sigwart[4]
[1]Oxford University Museum Of Natural History, Oxford, United Kingdom, [2]Museum für Naturkunde, Berlin, Germany, [3]Lund University, Lund, Sweden, [4]Queen's University Marine Laboratory, Portaferry, Northern Ireland

Vision and photoreception in echinoderms have perplexed researchers for more than a century. Recent experiments and novel techniques have revealed not only large numbers of opsin genes and photoreceptors, but the capacity for image formation in sea stars and urchins. Ophiocoma wendtii is a common Caribbean brittle star that exhibits dramatic photoresponsive colour changes and strong negative phototaxis. It has become iconic in vision research, speculatively possessing a unique whole-body compound eye. Hemispherical calcite structures on the aboral surface putatively focus incoming light onto sensory nerve bundles beneath, which could be integrated to allow spatial resolution. However, no photoreceptor cells were identified, and behavioural studies have been limited, despite widespread references to O. wendtii's visual ability. We present the first definitive description of an extensive extraocular photoreceptor network capable of spatial resolution in three species of Ophiocoma. Histology, immunolabelling and synchrotron tomography demonstrate that thousands of putative photoreceptors are likely responsible for Ophiocoma's photosensitivity; however, the role of the 'lenses' has been misinterpreted. Behavioural experiments showed that two species were indeed able to detect large shapes from a distance and move towards them, indicating that they are capable of integrating activity from thousands of photoreceptors to contribute to an elementary form of spatial vision. Results suggest that pigmentation and chromatophore activity determine the presence or absence of visual behaviour, and not the structures previously interpreted as lenses. Although some species are able to use this distributed photoreceptor network for spatial resolution, the underlying mechanism for signal integration remains elusive.

[S3-4] Gaze control when having plenty of eyes and little brain power

Dr Anders Garm[1], Mr Sofus Wiisbye[1]
[1]University of Copenhagen, Copenhagen Ø, Denmark

It is important for all visually guided animals to know where in the surroundings the visual input comes from and thus they must have some information about the gaze direction of the eyes. This can be challenging not least for animals with dispersed visual systems containing many eyes and often limited nervous system. A good example of such a dispersed system is found in cubomedusae which have four sensory structures, rhopalia, each holding six eyes of four types. The rhopalia hang by a flexible stalk and a heavy crystal in the distal end act as a weight ensuring that the rhopalia are always upright no-matter the orientation of the medusa. In this way the vertical part of the visual field is kept constant for all the eyes. We have investigated the horizontal orientation of the rhopalia by spinning horizontally oriented medusae in a computer controlled LEGO device. It showed that the horizontal part of the visual field is also strictly controlled and determined by the position of the rhopalium in the rotational cycle. This is a consequence of the mechanical properties of the rhopalial stalk but the orientation is fine-tuned by the visual input. There is a pair of pit eyes on each rhopalium and if they are stimulated differently they activate muscles in the stalk turning the rhopalium in the horizontal plan. The medusae thus have complete control of the gaze direction of all their 24 eyes.

Plenary Lecture 2

[PL2] Odor evoked activity across brain layers: codes, patterns and memories

Professor C. Giovanni Galizia[1], Dr Alja Lüdke[1], Dr Georg Raiser[1], Dr Paul Szyszka[1]
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Most odors are encoded by combinatorial activity across neurons, in sequential neural layers: olfactory receptor neurons (vertebrate nose, insect antennae), olfactory glomeruli (olfactory bulb, antennal lobe), higher order brain centers (piriform cortex, mushroom body). Olfactory stimuli elicit complex spatio-temporal patterns of neural activity. What is the readout? Does the brain need the full spatio-temporal pattern to recognize an odor, or is a snapshot sufficient? Learning experiments show that odors are recognized almost immediately at stimulus onset, also when during training they are learned later or even after stimulus offset. This suggests that different time windows fulfil different tasks, or are used in different ways by the brain. We have analyzed the temporal development of odor information in different neuron populations in the fruit fly Drosophila melanogaster: receptor neurons, projection neurons in the antennal lobes (dendrites and somata), and Kenyon Cells in the mushroom bodies (dendrites and somata). When measuring calcium concentration changes, odor information was stable during odorant presentation both for short and long stimuli, and changed after odor offset, providing reliable information about both odorant onset and offset. For a subset of Kenyon cell somata, however, we found ongoing odor information for several seconds beyond odor offset, suggesting a neuronal substrate for sensory memory that could be used in trace conditioning situations, i.e. when a reward arrives after stimulus offset. We show that it is necessary to analyze subcellular compartments: information across cell bodies has different temporal progression (and information content) than across dendrites, possibly indicating multitasking within cells.

Oral Session 1

[OR1] Parallel and serial visual search modes in the archerfish

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Visual search is the ability to detect a target of interest against a background. For many animals, preforming this task fast and accurate is crucial to ensure survival. The difficulty of a visual search task depends fundamentally on the target's and distractors features and on their interaction. Substantial effort was devoted to investigate the influence of many different features on the visual search performance of human, and many visual processing models were suggested. Yet, little is known about the visual search ability of other vertebrates. In this study we investigated the visual search performance of the archerfish, a non-mammalian vertebrate that lives underwater and lacks a fully developed cortex. We found that the archerfish can carry out parallel and serial visual search modes. We found that color, size, motion and orientation features elicit the target to "pop-out" and resulted in parallel search mode in the fish. In contrast, shape feature elicits serial search mode. Finally, we tested conjunction search task where the target was characterized by a unique combination of color and size and found asymmetry in the task performance. Interestingly our results are comparable with what was found in humans and suggest that there is universality in the way visual search is done by animals with very different brain anatomy.
Keywords: Visual-search; reactions-times; serial search.

[OR2] Stereo vision and second order motion sensitivity in the praying mantis

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Praying mantises are the only insects known to have stereo vision. We recently showed that, unlike primates and birds, stereo vision in mantises is not affected by mismatches in the stationary luminance patterns viewed by both eyes. Instead, it is sensitive to changes in the patterns at the appropriate parallax across the two eyes. This makes it sensitive to second-order motion but the mechanisms by which mantis stereo vision exploits second-order motion are unknown. We therefore ran a series of experiments in which we presented mantises with stimuli with different combinations of first and second order motion cues and tested them for their depth perception in these stimuli. Our results show that second order motion enables figure detection which then leads to stereoscopic depth detection by mantises. Stereopsis in mantises is thus fundamentally dependent on second-order figure motion and not first-order elementary motion of the details that make up the figure.

[OR3] Optic flow alters regional visual acuity and attention in flying fruit flies

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For a moving animal, vision provides instantaneous information about course, speed, and heading, but motion simultaneously degrades the visual signal itself. Fast moving images reflect few photons before slipping over a new position on the retina, and when photons are scarce, images suffer from low signal to noise ratios. A well known optical adaptation of fast flying insects is the equatorial increase in interommatidial angle between the front and side of the eye. This creates a region that collects more photons at the expense of acuity, but only in lateral directions where fast optic flow is common. Drosophila eyes however, display only mild changes in lateral interommatidial angle, implying they risk information loss during forward flight unless they can compensate with neural mechanisms. To determine how flies process visual signals degraded by motion blur, we exposed tethered flying fruit flies to forward optic flow and used local steering cues to test their regional acuity and attention. We found that flies reduce resolution in regions of fast motion, but only parallel to the direction of motion, and favor spatial filtering, to retain the fast temporal responses necessary for fast motion detection. Additionally, flies shift attention to forward regions in the optic field, where optic flow is typically slower. These strategies may maximize visual information by preserving lateral acuity when a fly is still, but improving sensitivity during fast flight.

[OR4] From visual processing in the optic tectum of barn owls to Japanese optical art

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The Ouchi illusion, named after the Japanese artist Hajime Ouchi, is an illusion by which a disk whose texture is oriented differently from its background is perceived as if moving relative to background. The illusion, believed to rely on the optic flow generated by small eye movements, is elevated if the display is moved from side to side. Barn owls, which lack the capability for prominent eye movements, occasionally perform peculiar side to side head motions (peering) when scanning the environment. Here, we first measured natural peering trajectories. Later, we recorded, in head fixed owls, neural responses to visual displays shifted along trajectories computed to induce relative motion on the retina as in the peering motions. We presented wide-field displays of densely packed stripes with a dominant orientation. Visual objects were created by orienting a circular patch differently from the background. We found that moving the visual display (object with background) along the computed trajectories induced dramatic neural responses to the objects, in neurons that where unresponsive to the objects in static displays. The analysis of the responses generated two predictions regarding the illusionary motion: 1. the perceived direction of motion will be shifted by 90^0 if the dominant orientations of the object and background are swapped. 2) the perceived direction of motion will be determined by the background orientation relative to motion direction. We confirmed both these predictions in humans observing a variant of the Ouchi illusion. Thus, peering motions may facilitate orientation based camouflage-breaking. Moreover, similar neuronal mechanisms likely induce the apparent motion of the Ouchi illusion.

[OR5] Multiple rod opsin-based vision in deep-sea fishes

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Vision in vertebrates is based on different visual proteins (opsins) in the cone and rod cells in the retina. Under ‘dim-light’ conditions, mostly rod receptors are thought to mediate rather color-blind vision by expression of a single rod opsin gene (RH1), while the cones enable colour vision in substantial light intensity. By inspecting 101 fish genomes, we found that three teleost lineages from the dim-light environment of the deep sea have independently expanded their RH1 gene repertoire via gene duplication and subsequent functional diversification. An extreme case of the silver spinyfin (Diretmus argenteus) stands out with a total of 40 opsin genes in its genome (2 cone + 38 rod opsins), and has the highest number of visual opsins known for animals so far. We found that 14 RH1 genes are simultaneously expressed in the morphologically unique retina of D. argenteus. The in-vitro synthesis and functional prediction revealed that these genes encode for photopigments with different spectral sensitivities (λmax spanning 65 nm), covering efficiently the range of the residual daylight in the deep sea, as well as bioluminescence emitted from deep-sea organisms. By additional modeling, we tested for the putative function of such unique set-up: does such system serve to colour vision, or rather to boost sensitivity within the entire light spectrum in the depth? In any case, we present the first molecular evidence for exclusive multiple (>3) rod-opsin-based vision among vertebrates, and not surprisingly such system has been discovered in the fascinating deep-sea fishes constantly challenged by their extreme environment.

[OR6] Structural, molecular, and functional complexities of the distributed visual systems of chitons

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Chitons (Mollusca: Polyplacophora) display a diversity of sensory structures, called aesthetes, embedded within their eight overlapping shell plates. All chitons have non-pigmented clusters of multimodal sensory cells called megalaesthetes. In addition to these, some chitons have small eyes with image-forming lenses. Still other chitons have modified megalaesthetes that include an eyespot. To ask how eyes and eyespots may have evolved from megalaesthetes, we examined the relationships between the molecular, structural, and functional complexities of these sensory organs. We studied molecular and structural complexity by comparing the expression of molecular components of sensory transduction in chitons that represent each character state: a chiton with megalaesthetes, a chiton with eyespots, and a chiton with eyes. We learned that megalaesthetes express many molecular components of sensory transduction; that the retina-like structures of eyespots express a subset of the proteins found in megalaesthetes; and that the photoreceptors in the eyes express fewer molecular components of sensory transduction than megalaesthetes and eyespots. To address the relationship between structural and functional complexity, we tested how chitons with eyespots respond to changing overhead stimuli and static visual landmarks. Like all chitons, those with eyespots respond to changes in the overhead light field. In contrast to chitons with eyes, chitons with eyespots do not distinguish between objects and equivalent, uniform changes in light levels. However, we found that chitons with eyespots have the ability to orient to static, lateral visual landmarks. Though eyespots appear to represent structural and molecular intermediates between megalaesthetes and eyes, they do not represent functional intermediates.

[OR7] Low resolution vision in a velvet worm (Onychophora), a close relative of arthropods that resembles Cambrian fossils

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Onychophorans, also known as velvet worms, are bizarre, soft-bodied animals of few centimetres in length with multiple legs, two antennae, and the ability to shoot glue from two slime papillae on their head. While their appearance is in fact velvety, they are not worms. Instead, they resemble Cambrian fossils and are close relatives of arthropods, which boast an unrivalled diversity of eye designs. Because of their phylogenetic position and similarity to ancient forms, onychophorans are a key lineage for understanding the evolution of vision. Nonetheless, the visual capabilities of onychophorans have not been well explored. We assessed the spatial resolution of the onychophoran Euperipatoides rowelli using behavioural experiments, three-dimensional reconstruction, anatomical and optical measurements, and modelling. Exploiting an innate attraction towards dark objects, we find that E. rowelli can resolve stimuli that are isoluminant with their background. Spatial resolution is in the range of 15° to 40°, which results from an arrangement where the cornea and lens project the image largely behind the retina. The peculiar ellipsoid shape of the eye and asymmetric position of the lens may improve spatial resolution in the forward direction. Yet by itself, the unordered network of interdigitating photoreceptors, which fills the whole eye chamber, excludes high acuity vision. Our results suggest that E. rowelli cannot visually identify prey or conspecifics beyond about 1 cm from the eye. However, their coarse spatial resolution could aid them in aiming the glue spray towards a prey or looming predator at close distance, and is likely sufficient to find shelter and suitable microhabitats.

[OR8] The evolution of true colour vision across jumping spiders

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In many animals, vision plays a central role in navigation, foraging, and communication. The diversification of visual systems is thus important for exploitation of new visual niches. Jumping spiders are visually guided predators with principal eyes that provide high spatial acuity and colour vision. They also exhibit major differences in colour signalling across species, particularly in the use of long-wavelength colours in male courtship displays. Given that most jumping spiders are thought to have UV-green dichromatic colour vision, which should not allow perception of long-wavelength colours, we hypothesized that jumping spider groups that use such colours in communication may have evolved improved colour vision. Within a comparative framework, we investigated the number and peak sensitivities of photoreceptor types in the principal eyes of jumping spiders using microspectrophotometry. We identify one origin of trichromacy, in the Harmochirines and Pellenines (which include the colourful genus Habronattus). We also report two independent origins of tetrachromacy, one in the Euophryini (which includes the Maratus peacock spiders) and a second in the Aelurillina (in the colourful genus Stenaelurillus). Trichromacy in Harmochirines/Pellenines is achieved using spectral tuning via an intraretinal long-pass filter, whereas both instances of tetrachromacy are achieved by the addition of photoreceptors with different spectral sensitivities. Jumping spiders thus represent a promising group for the study of repeated evolution of true colour vision in terrestrial habitats. We discuss the potential role of predatory behaviour, sexual selection, light conditions and background complexity in the context of evolution of colour vision in this group of spiders.

Oral Session 2

[OR9] Neuropeptide F drives attentional gain in the fly brain

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Gain control theory proposes that behavioral choices follow stimuli that evoke stronger responses in the brain. Here, we use a closed-loop choice paradigm to test this in Drosophila melanogaster. We first probe the flies' innate visual preferences, and find a robust preference profile for the size of objects. By recording brain activity via local field potentials (LFPs) in behaving flies, we find that attraction and repulsion to different-sized objects is correlated with neural gain in a central brain structure, the fan-shaped body (FB). Optogenetic activation of Drosophila neuropeptide F (dNPF) -expressing neurons, which innervate the FB, can render repulsive objects more attractive by increasing their neural gain. This suggests that dNPF controls visual behavior in Drosophila by regulating the relative gain of competing stimuli being processed in the FB. Simultaneous whole cell patch clamp recordings of a subset of dNPF neurons (P1 and P2), reveal how these neurons might control attentional gain in the fly brain.

[OR10] Specific octopaminergic neurons arbitrate between perseverance and reward in hungry Drosophila

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In pursuit of palatable food, hungry animals mobilize significant energy resources and overcome obstacles, exhaustion and fear. Their perseverance depends on metabolic state, internal motivation and the expected benefit. Sustained commitment to a trying task is crucial, however, disengagement from one behavior to engage into another can be essential for optimal adaptation and survival. How neural circuits allow prioritizing perseverance over withdrawal based on the animal's need is not understood. Using a single fly spherical treadmill, we show that hungry flies display increasing perseverance to track a food odor in the repeated absence of the predicted food reward. While this perseverance is mediated by a group of dopaminergic neurons, a subset of neurons expressing octopamine, the invertebrate counterpart of noradrenaline, provide reward feedback and counteract dopamine-motivated food seeking. Anatomical and physiological data show that these octopamine neurons are activated by sugar and connect the primary taste area to higher cognitive regions including the mushroom body network. Together our data suggest that a specific subset of octopaminergic neurons regulates the switch between two antagonistic behaviors, goal seeking and goal finding, by integrating internal state-dependent motivation with finding of the desired reward.

[OR11] The contribution of voltage-gated sodium channels to sensory neuronal excitability during hibernation

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During hibernation, thirteen-lined ground squirrels reduce their body temperature to ambient levels, which can be as low as 4°C. While many mammals perceive temperatures below 10°C as painful, squirrels tolerate cold in both the active and torpid states. Squirrels require adaptations to reduce cold sensitivity during hibernation, while retaining basal neuronal function at cold temperatures. Although most neuronal parameters, such as resting membrane potential, input resistance, and rheobase, are unchanged by torpor, somatosensory neurons from torpid squirrels have reduced firing rates. Furthermore, the firing patterns of torpid neurons are less robust, shifting their distribution from tonic firing, to irregular and single firing. We tested whether voltage-gated sodium channels influence this change in excitability. Three sodium channel subtypes are critical for the function of pain-sensing neurons by initiating and propagating action potentials: Nav1.9, Nav1.8, and Nav1.7. Interestingly, we found that each channel contributes to reduced excitability during torpor in a different way. Nav1.9 has shifted inactivation kinetics leading to fewer available channels. Tetrodotoxin-sensitive current densities, conducted in part by Nav1.7, are reduced by half. Nav1.8 activation is right shifted, making it harder to activate, and inactivation is slower. We are now working on a model to understand the interaction of these channels within the same cell, and to predict whether other channels also contribute to the reduced firing rates in torpid neurons. Our research suggests that changes in the function of voltage-gated sodium channels can decrease the excitability of sensory neurons and thus contribute to tolerance of extreme cold during hibernation.

[OR12] Visual surprise promotes sleep in Drosophila

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Sleep is a fundamental process observed in most animal species, yet we still know little about what drives the need to sleep, and the neural mechanisms involved. In this study, we used the fruit fly, Drosophila melanogaster, as a model to understand how visual processing affects sleep. We discovered that a classical motion blind mutant - optomotor blind – sleeps much less than wild type flies. Interestingly, apart from its known defects in horizontal system (HS) and vertical system (VS) neurons (which respond to lateral motion and drive the optomotor reflex), we found that optomotor blind mutants also lack a particular subset of loom sensitive neurons. To tease apart which neurons might be affecting sleep, we optogenetically activated different subsets of visual neurons in the fly brain, and monitored the effect on sleep during and following activation. Interestingly, activation of neurons in the loom sensitive pathway dramatically increased sleep, whereas activation of HS and VS motion sensitive neurons had no effect. On the other hand, optogenetic inhibition of loom sensitive neurons had little effect on sleep, suggesting the accumulation of sleep pressure is an active process. This suggests that distinct visual pathways are involved in the accrual of sleep pressure. Since loom stimuli evoke ‘fear-like’ responses in flies, whereas lateral motion stimuli do not carry value, we hypothesise that sleep is regulated specifically by visual stimuli that are salient and value-laden.

[OR13] The organization of projections from olfactory glomeruli onto higher-order neurons in Drosophila

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Different odors can evoke strikingly different behavioral responses, often without any prior experience. How does the brain organize the broad diversity of odors to ultimately support innate behavior? Individual odors typically activate multiple glomeruli and individual glomeruli are typically activated by multiple odors, forming a combinatorial code. downstream circuitry reads out this combinatorial code is incompletely understood. In the fruit fly, Drosophila melanogaster, glomerular projections target the mushroom body and lateral horn, two higher-order olfactory regions. The lateral horn is implicated in innate olfactory behavior and, consistently, projections to the lateral horn are stereotyped across animals. However, the logic of connectivity between glomeruli and lateral horn neurons (LHNs) is unclear. To uncover connectivity with single glomerulus and single LHN resolution, we combined two-photon optogenetics with whole cell patch-clamp electrophysiology. We have systematically mapped the glomerular inputs to ~40 morphological types of LHNs and found that different types receive input from different glomerular combinations. Thus, the connectivity of different glomeruli onto LHNs is not specified independently. Glomeruli that wire together do not typically respond to the same odors, but instead respond to odors with a similar natural ecology or behavioral relevance. Convergent glomeruli therefore define odor patterns with ethological importance. LHN types that receive selective input from certain glomerular groups project to specific brain regions beyond the lateral horn involved in multimodal integration, navigation, and learning. The odor tuning of each glomerular family and the projections of its cognate LHNs begin to define new roles for higher brain regions in specific olfactory behaviors.

[OR14] In vivo 24-hr whole-brain calcium imaging revealing how Drosophila circadian clocks regulate diverse behavioral rhythms

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Animals have circadian rhythms in a variety of physiological functions and behaviors, such as locomotor, sleep, and mating behaviors. In Drosophila melanogaster, these behavioral rhythms are driven by circadian clock genes oscillating in ~150 circadian pacemaker neurons. To explain how pacemaker neurons encode time and regulate different behavioral rhythms, we performed whole-brain calcium imaging in vivo for 24 hours using light-sheet microscopy. Firstly, we found that five major groups of pacemaker neurons display synchronous clock gene oscillations, yet each exhibits a distinct phase of daily neural activation. The activation phases of pacemaker groups that were associated with the morning or evening locomotor activities occurred ~4 hours before their respective behaviors. We further asked how synchronous clock gene oscillations generate nonsynchronous multi-phasic neural activity pattern. The pacemaker neural activities with a proper phase pattern required external light inputs and internal inhibitions between pacemakers. Light and inhibitory neuropeptides PDF and sNPF act dynamically at distinct hubs of the circuit to produce several multi-hour delays that create the proper tempo and sequence of pacemaker neural activities. Finally, we asked how pacemaker neural activities regulate different behavioral outputs. The circadian regulations were mediated by several downstream output circuits. These output circuits showed pacemaker-driven circadian rhythms in their neural activities that were associated with locomotor, sleep, and mating behavioral rhythms.

[OR15] Perceptual and neural limits of olfactory processing speed in Drosophila

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The brain encodes information by the rate and timing of action potentials (spikes) across neurons. Sensory systems often use temporal codes which rely on millisecond precise spike timing patterns. In olfaction, however, such precise spike timing has not been considered to be of importance for encoding odors, and the temporal precision of stimulus-evoked spikes in olfactory receptor neurons has not yet been accurately determined. Here we test the speed of olfactory processing in Drosophila: 1) We show in behavioral experiments that flies can use stimulus onset asynchrony to separate odorants from different sources. 2) Using simultaneous recordings from two olfactory receptor neurons of the same type, we demonstrate hitherto unknown fast and precise odorant-evoked spike responses, with first-spike latencies as short as 3 ms and with a trial-to-trial standard deviation (jitter) of only 0.15 ms. 3) We test the plausibility of a rapid, concentration-invariant latency code for odorant identity in a neural network model of the insect olfactory system.
These data provide new upper bounds for the speed of olfactory processing and suggest that the insect olfactory system can use the precise spike timing for olfactory coding and computation, which could explain insects' rapid processing of temporal stimuli when encountering turbulent odor plumes.

[OR16] Neuroarchitecture of the Drosophila Central Complex: A GAL4-Based Analysis of Nodulus and Asymmetrical Body Neurons

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The central complex, a set of neuropils in the center of the insect brain, integrates multimodal information about the external world and the animal's internal state and generates appropriate behavioral responses. Stereotyped neurons interconnect these neuropils with one another and with accessory structures. We searched 8200 Drosophila melanogaster GAL4 lines for expression in two neuropils, the noduli (NO) of the central complex and the asymmetrical body (AB), and used multicolor stochastic labelling to analyze the morphology, polarity, population size and organization of individual cells in a subset of the GAL4 lines that showed expression in the NO and AB. Identified NO and AB cell types will be discussed. The NO neurons receive input primarily in the lateral accessory lobe and send output to the NO. We demonstrate that the AB is a bilateral structure which exhibits asymmetry in size between the left and right bodies. We show that the AB neurons directly connect the AB to the central complex and accessory neuropils, that they target both the left and right ABs, and that one cell type preferentially innervates the right AB. We propose that the AB be considered a central complex neuropil in Drosophila.

Oral Session 3

[OR17] Neural and behavioral responses to communication signals across three apteronotid species: the influence of social group size on chirp discrimination.

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Communication signals mediate social interactions, and species that have more frequent social interactions may have a greater need to accurately encode and perceive variations in communication signals. We test this hypothesis by comparing behavioral and sensory responses in three species of apteronotid weakly electric fish to conspecific communication. We first quantify gregariousness to show that one species (A. devenanzii) prefers to group, one (A. albifrons) prefers to be solitary and a third (A. leptorhynchus) is in between. The propensity to use communication signals varies accordingly. Using a habituation-dishabituation paradigm we show that discrimination of chirps also varies across the three species. The more social species discriminate between chirps more accurately than the solitary A. albifrons. The analysis of neural responses in the electrosensory lateral line lobe (ELL) indicates that sensory encoding accuracy reflects these behavioral differences. We examine the neural properties of these pyramidal cells of the ELL to identify differences in the basic response characteristics of the neurons that can explain differences in chirp coding performance. We show that, although neural properties are generally similar across species, relevant differences exist such as frequency tuning and burstiness. Our study suggests that the evolution of social group size and communication propensity is linked with corresponding changes in the accuracy with which communication signals variations are encoded early in the sensory system.

[OR18] Dynamic circuit activity within a social salience neural network during social bond formation in the monogamous prairie vole

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Pair bonding is a social behavior that has evolved in ~5% of mammalian species, including humans. The neurochemical mechanisms of pair bonding have been extensively studied, revealing critical roles for oxytocin, vasopressin and dopamine acting on nodes within a social salience neural network. Simply knowing the sites of action, however, does not tell us how these brain areas are actually engaged during the social interactions that establish a pair bond. We are investigating this question in the monogamous prairie vole (Microtus ochrogaster) model of social bonding using in vivo recordings of local field potentials from the nucleus accumbens (NAcc), medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) during a period of cohabitation between male and female voles. Our working hypothesis is that functional connectivity between these brain areas mediates learning about the rewarding nature of social interactions with an emerging partner, increasing the salience of partner cues, and driving more affiliative behavior with the partner. In non-social learning paradigms, the mPFC-NAcc circuit helps predict the rewarding outcome of a cue to elicit seeking behaviors, and results from our electrophysiological and optogenetic stimulation studies demonstrate a similar role for this circuit in the female prairie vole brain during cohabitation (Amadei, Johnson et al, Nature, 2017). I will present those results within a more general theoretical framework of how the NAcc may be integrating different types of social information from mPFC, BLA and ventral hippocampus to help animals switch their behavioral response to partner cues, facilitating affiliative while suppressing non-affiliative behaviors with the partner.

[OR19] Social status-dependent regulation of the dopaminergic system and regulation of spinal motor circuits in zebrafish (Danio rerio)

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We investigated the neural bases of social regulation using zebrafish as a model organism. Zebrafish form stable hierarchies that consist of socially dominant and subordinate fish. In addition to swimming behavior, zebrafish produce an escape response mediated by the Mauthner neurons. Using a non-invasive technique we recorded the activation of the escape and swim circuits in the two social phenotypes. We show social-status dependent effects in the activation of the two competing neural circuits. Subordinates favor escape over swim while dominants favor swimming over escape. To test the role of dopamine in regulating these differences in motor patterns, we augmented dopamine levels. As a result, differences in escape behavior between the two social phenotypes were diminished suggesting a social status-dependent regulation of dopamine of the escape circuit. Further, whole brain gene expression analysis showed significant upregulation of the dopamine transporter (dat) in dominants and downregulation of the dopamine receptor 1b (drd1b) in subordinates. We investigated the role of drd1b expression by pharmacologically injecting specific drd1b agonists and antagonists. Blockage of the drd1b shifted dominant behavior from favoring swim over escape to escape over swim; thus, resembling subordinate behavior pattern. In addition, antagonizing drd3 lowered subordinates' probability of escape with minimal effects observed on dominants. Taken together, these results suggest that an animal's social status can shift the activation of competing neural circuits mediated, in part, through the balance of dopamine supply regulated by dat and interpretation of dopamine by differences in drd1 and drd3 expression.

[OR20] Oxytocinergic regulation of adult social behavior in zebrafish: developmental effects and signalling pathways

Professor Rui Oliveira[1], Dr Rita Nunes[2], Mr Michael Gliksberg[3], Dr Savani Anbalagan[3], Dr Einav Wircer[3], Dr Janna Blechman[3], Professor Gil Levkowitz[3]
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Oxytocin-like nonapeptides (isotocin in fish and oxytocin in mammals) have been implicated in the regulation of social behavior across vertebrates. Although vasopressin has been more involved in aggression behavior, whereas oxytocin has been mainly implicated in pro-social behaviors, these nonapeptides and their receptor subtypes are similar and so, most of the classic pharmacological evidence does not differentiate their specific contribution to different social behaviors. In this study we used zebrafish, a vertebrate model organism with a well-characterized repertoire of social behaviors and a broad genetic toolbox available, to investigate the role of oxytocin-like peptides on social behavior. For this purpose, we developed genetic tools (GAL4-UAS lines) to manipulate the oxytocinergic neuronal circuits and to study how loss of function of these neurons during development, affects the development of social behavior. Our results indicate a role for oxytocinergic neurons in the acquisition of zebrafish sociality, a trait that emerges during the third week of development. A conditional and cell-specific ablation of these neurons at a critical developmental time window, but not during adulthood, significantly altered specific adult social behaviors in zebrafish, suggesting an unique organizational, rather than activational, effect. Furthermore, using genome-editing methods (i.e. TALEN, CRISPR), we found evidence that oxytocinergic neurons may modulate different aspects of social behavior (motivational vs. cognitive) through different signaling pathways.

[OR21] Fluctuations in abundance cause changes in the cognitive performance of cleaner fish Labroides dimidiatus

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The marine cleaning mutualism involving the cleaner fish Labroides dimidiatus provides a model system to study the potential links between cooperation and cognition. Cleaners are known for their sophisticated social strategies during interactions with 'client' reef fishes, raising questions about the underlying cognitive processes. Recent extreme weather events at Lizard Island, Great Barrier Reef allowed us to test how changes in supply and demand affect the cleaners' performance in laboratory cognition experiments: cyclones and El Niño reduced cleaner densities by 80%, disproportionally to the reduction in client densities. We found a significant decline in the ability of cleaners to manage their reputation and to learn to prioritise ephemeral food sources to maximise food intake in laboratory experiments. In other words, cleaners failed to display the previously documented strategic sophistication that made this species a prime example for an intelligent fish. It turned out that in nature, client demand for cleaning had increased and clients were more willing to wait and allow inspection. Therefore, cleaners had apparently learned to adjust, abandoning the sophisticated strategies that would have been needed to solve the experimental tasks. In line with this interpretation, performance improved again in 2017, together with an increase in cleaner population densities. In conclusion, cleaner strategic sophistication is most likely based on associative learning according to the local cleaning market conditions.

[OR22] Neurobiology of pair bonding in fishes: convergence of neural mechanisms across distant vertebrate lineages

Dr Jessica Nowicki[1], Prof. Morgan Pratchett[2], Dr. Stefan Walker[2], Dr. Darren Coker[3], A. Prof. Lauren O'Connell[1]
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Pair bonding is a spectacular evolutionary innovation that has independently evolved numerous times among vertebrates. The neural mechanisms of pair bonding have been studied in depth in the mammalian model species, the prairie vole, Microtus ochrogaster. In this species, oxytocin (OT), arginine vasopressin (AVP), dopamine (DA), and opioid (OP) systems play key roles in the formation and maintenance of pair bonding by targeting specific social and reward-mediating brain regions, including the lateral septum and striatum. By contrast, the neural basis of pair bonding is poorly studied in other vertebrates, and especially those of early origins, limiting our understanding of the evolutionary history of this fascinating social system. Using a novel teleost model, Chaetodon lunulatus, we compared receptor gene expression between pair bonded and solitary individuals across eight socio-functional brain regions. We found that in females, OT-like and AVP-like receptor expression varied in the lateral septum-like region (the Vv/Vl), while in both sexes DA-1, DA-2, and mu-opioid (MO) receptor expression varied within the mesolimbic reward system, including a striatum-like region (the Vc). Our results overall mirror patterns found in M. ochrogaster and provide novel insights into the neurobiology of teleost pair bonding. Our findings suggest that neural mechanisms of pair bonding have somewhat converged between teleosts and mammals, by repeatedly co-opting and similarly assembling deep neurochemical and neuroanatomical homologies already present in a common ancestor ~450 million years ago.

[OR23] Numerical cognition in bees

Miss Scarlett Howard[1], Dr Aurore Avarguès-Weber[2], Dr Jair Garcia[1], Professor Andrew Greentree[1], Professor Adrian Dyer[1]
[1]RMIT University, Melbourne, Australia, [2]Université de Toulouse, Toulouse, France

Many non-human animals demonstrate some level of numerical ability which includes an understanding of complex number concepts such as addition, sequential ordering of numerical elements, or even the concept of zero. Although very little research has been done on numerical ability in invertebrates, honeybees and several other insects have been shown to possess some numerical capabilities. We will discuss our recent findings regarding honeybees and their ability to process number. We assessed the capacity of bees to complete numerical problem-solving tasks achieved by primates and other vertebrates. These tasks included number categorisation, understanding and applying number rules, and learning to solve symbolic number problems. Honeybees were able to learn numerical rules and even extrapolate those rules to new numbers or tasks to demonstrate a flexible and complex acquired understanding of different numerical concepts. In some cases, honeybees have mastered numerical concepts at a level that parallels numerical abilities demonstrated by primates, mammals, birds, and other vertebrates.

[OR24] Biogenic amine modulation of honey bee sociability and nestmate affiliation

Miss Susie Hewlett[1], Miss Deborah Wareham[1], Mr Thomas Pyne[1], Dr Andrew Barron[1]
[1]Macquarie University, Sydney, Australia

The success of a society depends upon the coordinated and cooperative action of each member. For an individual to live in a cohesive group, they must be gregarious by nature and experience a social environment that promotes appropriate development. This drive to aggregate and interact with others is termed sociability. Here we combine an assay that simultaneously measures honey bee (Apis mellifera) sociability and a preference to affiliate with nestmates with ocellus injection of biogenic amines (BA) and their receptor antagonists, to explore which BA systems might modulate this behaviour. Bees injected with dopamine (DA) were consistently highly sociable. They spent the majority of the test time in proximity to conspecifics and were also more likely to interact with conspecifics than other treatment groups. Moreover, injection of the DA receptor antagonist, fluphenazine (flu), led to a lower sociability score than DA injected bees. Octopamine (OA) injection did not alter time with conspecifics compared to controls, but resulted in no interactions with conspecifics. DA injection increased affiliation with nestmates and flu injection had the opposite effect of more time spent with non-nestmates. The present study highlights comparable neural mechanisms behind sociability in mammals and honey bees at the neurochemical level. Continued investigation of mechanisms driving insect sociability will provide a better understanding of social evolution.

Franz Huber Lecture

[FHL] Thinking like a leech: A neuroethological view of behavioral choice.

Dr Bill Kristan[1]
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In 1951, Nikko Tinbergen proposed—based purely upon behavioral observations--that animals chose to perform one of many possible behaviors based upon “inhibitive interactions” among “centres” responsible for initiating a particular behavior. In the early 1970’s, Jack Davis and his colleagues, working on the marine slug, Pleurobranchaea, translated this notion into more specific neuronal terms: they showed inhibitory synaptic connections onto “command neurons” responsible for eliciting a particular behavior A by eliciting behavior B. These early studies also established the “competing behaviors” paradigm for studying behavioral choice: simultaneously deliver two stimuli, each of which produces a distinct behavior, then see which behavior wins out. More recent studies on other mollusks have produced mixed results. I will discuss two types of behavioral choice mechanisms that my laboratory has found, using leeches, one that shows a type of inhibitive interaction and a second that implicates different dynamical states of a complex decision-making network. As a graduate student, Quentin Gaudry studied how feeding behavior in the European medicinal leech predominates over mechanosensory-induced behaviors (crawling, shortening, and local bending). He did find inhibitive interactions, but they were not at the level of command neurons. Instead, the detection of food and the act of feeding produced profound presynaptic inhibition of the mechanosensory neurons all over the body. Effectively, when a leech detects a meal, it becomes numb to mechanosensory input because transmission across the first-order mechanosensory synapses is blocked. In studying the choice between swimming and crawling, Kevin Briggman found no evidence of inhibitive interactions. Instead, he found that the same neurons—including command neurons--are activated by stimuli that lead to both behaviors, but these neurons were activated in different patterns when swimming was activated than when crawling occurred. This result suggests that this behavioral choice is made by the dynamics of the same neuronal system rather than by inhibition among behaviorally appropriate command neurons. Why should there be such different mechanisms of behavior choice in the same nervous system? One reason seems to be differences in lifestyle: feeding inhibits mechanosensory-induced behaviors only in sanguivorous leeches (for whom blood meals are rarely available, and they gorge themselves when they have the opportunity) and not in carnivorous leech species (which take small meals of more readily-available sources). Another reason may be evolutionary and functional: swimming behavior probably evolved from crawling behavior and these leech nervous systems may have kept their overlapping circuitry so that they can switch readily between the two locomotory behaviors. Hence, thinking like a neuroethologist—and like a leech—can point experimentation in useful directions.

Plenary Lecture 3

[PL3] Bees, Birds and Flying Machines

Professor Mandyam Srinivasan[1]
[1]University of Queensland, St. Lucia, Australia

Flying insects and birds are remarkably adept at seeing and perceiving the world, and navigating effectively in it. This presentation will describe our recent progress in understanding how honeybees and birds (Budgerigars) use their vision to guide and control several aspects of their flight such as regulating flight speed, negotiating narrow passages, selecting routes, and avoiding mid-air collisions, using computational principles that are often elegant and unprecedented. It will conclude with an update of our advances in the design and testing of biologically inspired vision systems for the guidance of autonomous aerial vehicles.

S4: Genomics-enabled approaches to neuroethology

[S4-1] The genomic basis of communication signal variation in electric fish

Dr Jason Gallant[1]
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Molecular variation contributes to the evolution of adaptive phenotypes, particularly behavior. Given the complexities of many behaviors, it is often difficult to understand precisely how. The electric organ discharge (EOD) behavior of weakly electric fish is the direct result of biophysical membrane properties set by ion channels and developmental processes that shape the flow of current through the electric organ. Two behaviorally salient, yet highly variable, aspects of EODs are duration and complexity. A recent proliferation of new genomic resources, including a mormyrid genome, has enabled unprecedented opportunities to identify the precise molecular underpinnings of EOD signal variation among species of weakly electric fish. First, I will focus on genes that contribute to the evolution of signal duration by describing a collabroative project where we examined voltage-gated potassium channel genes in African mormyrid electric fishes. We identified a particular gene that is highly expressed in the electric organ, is under positive selection within pulse-type mormyrids, and that exhibits unique biophysical properties in vitro that likely contribute to the evolution of short EODs.
Second, I will focus on our efforts to characterize genes that contribute to the evolution of complexity of mormyrid EODs. This work leverages previous studies of geographic variation and polymorphism in the mormyrid Paramormyrops kingsleyae against large RNA-seq and whole-genome resequencing datasets to identify developmental genes in mormyrids that influence the shape of electrocytes. To date, we have identified gene expression changes that correlate with changes in EOD complexity and are progressing toward identifying causative genomic loci. Supported by National Science Foundation #1455405, # 1557657 and #1644965

[S4-2] Variability, plasticity, and identity of neurons in "simple" behavioral circuits

Dr David Schulz[1]
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"Simple" behaviors such as locomotion are the result of highly robust neural circuits capable of generating reliable output over the lifetime of an individual. Each neuron involved must generate a precise pattern of firing, and do so in appropriate timing relative to all the other neurons of the circuit. Yet there is a growing appreciation that the underlying gene expression and physiology of the cells driving these behaviors is quite variable across individuals, despite highly convergent output. We are using the crustacean stomatogastric and cardiac ganglia to address links between gene expression, cellular physiology, and circuits that give rise to robust output. Single-cell RNAseq and comprehensive qPCR approaches reveal that there is no standard underlying blueprint for these cells. No two individuals seem to use the same combination of channels and receptors to come to a common output, but nevertheless get the job done. The only consistent feature we can identify across these networks are their connectivity and projection targets. This leaves us wondering whether even the most conserved behaviors and their underlying circuits represent emergent functionality from differentially tuned and re-tuned cells over the lifetime of an individual. Through this work, we are only beginning to understand the cellular processes that give rise to this phenomenon, as well as the implications of such variability for behavioral plasticity and evolution of circuits underlying these behaviors.

[S4-3] Adaptation to chemical defenses: molecular physiology of sensory-neuron TRP channels

Dr Ashlee Rowe[1], Dr. Matthew Rowe[1], Lauren Koenig[2], Olivia Gusweiler[2]
[1]University Of Oklahoma, Norman, United States, [2]Michigan State University, East Lansing, United States

Survival depends on responding appropriately to painful, potentially damaging stimuli. Transient Receptor Potential (TRP) channels transduce such stimuli. TRPA1 (Ankyrin) and TRPV1 (Vanilloid) are polymodal pain receptors triggered by a number of noxious chemicals, extreme temperatures, changes in pH and inflammatory tissue damage. However, TRPA1 and TRPV1 expression patterns, molecular mechanisms of polymodal activation, and role in inflammation are incompletely understood. Darkling beetles (Eleodes longicollis) and southern grasshopper mice (Onychomys torridus) provide an opportunity to examine the molecular physiology of TRP channels, and to explore changes in channel structure and expression impacting predatory behavior. Darkling beetles, when threatened, spray a chemical cocktail in their predator's face. One chemical, benzoquinone, activates TRPA1 causing irritation of the eyes and oronasal membranes in sensitive animals. Grasshopper mice (obligate carnivores) prey on darkling beetles, showing little response to beetle sprays, while Peromyscus (opportunistic insectivores) avoid the beetles. Two-bottle choice drinking tests and hind-paw injections show that grasshopper mice are less sensitive than house mice to chemicals that activate TRPA1 and TRPV1. To characterize changes in the structure and expression of TRP and related inflammatory genes that reduce pain signaling, we are comparing whole genome sequences and RNA transcriptomes of sensory tissue from grasshopper mice and other rodents. RNA-seq analyses of grasshopper mice sensory tissue revealed amino acid substitutions in TRPA1 and TRPV1. Sanger sequencing of TRPA1 confirmed modifications associated with chemical binding. Currently, we are producing a TRPA1 clone for heterologous expression to determine the functional consequences of channel modifications.

[S4-4] Cellular and molecular adaptations underlying different frequency regimes in rattlesnake spinal motor systems

Dr Boris P. Chagnaud[1]
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In vertebrates, locomotion is governed by highly conserved spinal circuits that generate patterned contractions of bilaterally organized skeletal muscles. While adaptations in the spinal blueprint circuit lead to different locomotor patterns (e.g. undulatory and bi- or quadrupedal patterns), individual motor patterns display widely diverging ranges of frequencies between fast or slowly moving animals. How these different ranges of frequencies are achieved remains enigmatic. Rattlesnakes offer a unique opportunity to address this question since they produce two different spinal behaviors at distinct frequency ranges. The predominant part of the cord generates low frequency alternating muscle contraction sequences for locomotion (few Hz), whereas the most caudal part of the spinal cord generates high frequency alternating patterns (80-120 Hz) for acoustic signaling (rattling). Using a variety of methods, including RNAseq of bulk tissue and single cells, we investigated the adaptations that enable these highly diverging frequency regimes. While the local neuronal circuitry appeared to be similar between the two cord regions (at the molecular and neurophysiological level), patch clamp recordings of spinal motoneurons involved in the rattle behavior revealed prominent differences for high frequency firing. Taken together, our results suggest that up or down regulation of specific ion channels might be sufficient to generate different frequency regimes, and in the case of the rattlesnakes even novel behaviors.
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S5: Vocal communication as a model for developmentally-regulated learning: An integrative approach

[S5-1] Neurobiological Investigation of Vocal Production Learning in the Mammalian Brain

Professor Michael Yartsev[1]
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Learning a language is generally considered the crown jewel of human abilities. Yet the core question of 'What is it about the human mammalian brain that allows us to learn our language?', remains unresolved. In humans, language acquisition is mediated by a process called 'vocal learning'. While humans are expert vocal learners, a remarkably sparse subset of mammals share this capacity and as a result, the neurobiological mechanisms of vocal learning were never studied before in the mammalian brain. To complement the remarkable research work done in the songbird and help bridge this major gap of knowledge we set out to establish the bat as a mammalian model system for studying the neurobiological mechanism of vocal learning. Here, I will present our initial efforts towards achieving this goal which included overcoming major roadblock due to the near complete absence of research efforts in this domain in mammals. These include (i) identifying the appropriate behavioral paradigms for studying the process of vocal production learning, (ii) the relevant neural circuitries which might mediate this process in the developing and adult mammalian brain and (iii) the establishments of the necessary novel technologies to support this new research direction.

[S5-2] Vocal learning via social reinforcement by infant marmoset monkeys

Dr Daniel Y. Takahashi[1,2], Diana A. Liao[1,2], Dr. Asif A. Ghazanfar[1,2,3]

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For over half a century now, primate vocalizations are thought to undergo little or no experience-dependent acoustic changes during development. If any changes are apparent, then they are routinely attributed to the passive consequences of growth. Indeed, previous experiments on squirrel monkeys and macaque monkeys showed that social isolation, deafness, cross-fostering and parental absence have little or no effect on vocal development. Here, we explicitly test in marmoset monkeys--a very vocal and cooperatively breeding species--whether the transformation of immature into mature contact calls by infants is influenced by contingent parental vocal feedback. Using a closed-loop design, we experimentally provided more versus less contingent vocal feedback to twin infant marmoset monkeys over their first two months of life, the interval during which their contact calls transform from noisy, immature calls to tonal adult-like “phee” calls. Infants who received more contingent feedback had a faster rate of vocal development, producing mature-sounding contact calls earlier than the other twin. The differential rate of vocal development was not linked to genetics, perinatal experience or body growth; nor did the amount of contingency influence the overall rate of spontaneous vocal production. Thus, we provide the first experimental evidence for production-related vocal learning during the development of a nonhuman primate.

[S5-3] A comparative approach to identifying mechanisms of socially guided vocal learning

Dr Michael Goldstein[1]
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Recent work has shown that contingent social responses to immature vocalizations in songbirds and human infants facilitate real-time vocal learning. What makes contingency effective? We hypothesized that social feedback is rewarding to young learners. Circuitry linking basal ganglia with cortical areas may integrate social reward with vocal control and may underlie socially guided vocal learning. We tested this hypothesis with studies in human infants and the zebra finch. We first used interactive video playbacks to assess the role of contingent feedback in song learning. Juveniles in the experimental condition received video playbacks of females exhibiting feather fluff-ups contingent on their own singing. These juveniles developed significantly better song matches to tutor song than did yoked controls. Next, to examine the neuroendocrine mechanisms involved in socially guided song learning, we manipulated the brains of young male zebra finch with argenine vasotocin (AVT) or Manning Compound (MC) so that they found social interaction more rewarding or less rewarding than controls. AVT birds learned song significantly better than MC birds, suggesting that vasotocin helps organize the reward circuitry that influences socially-guided vocal learning. Finally, we investigated whether social feedback was similarly rewarding for human infants. We used a conditioned place-preference paradigm in which social cues (infant-directed or adult-directed speech) were paired with different contexts. Infants developed a place preference for the infant-directed context. Taken together, these studies indicate that temporal characteristics of social interaction are rewarding to young songbirds and human infants, and reward pathways may drive learning in social contexts.

[S5-4] Cortico-basal ganglia circuits encode performance during goal-directed learning in juvenile songbirds

Dr Sarah Bottjer[1]
[1]University Of Southern California, Los Angeles, United States

“Procedural” skill learning involves goal-oriented evaluation of behavioral outcomes, which gradually shapes neural circuits to select appropriate actions. That is, acquisition of motor skills depends on neural circuits that compare feedback of self-generated movements to a desired goal and reinforce movements that match that goal. Neural circuits that traverse the cortex and basal ganglia are organized as recurrent loops that mediate diverse types of procedural learning. How they accomplish this feat is not well understood. Vocal learning in songbirds provides a powerful model for studying the control of experience-dependent skill learning by cortico-basal ganglia circuits during development. Songbirds, like humans, learn the sounds used for vocal communication during development. We investigated spiking activity in CORE and SHELL subregions of the cortical nucleus LMAN during development as juvenile zebra finches (Taeniopygia guttata) were actively engaged in evaluating feedback of self-generated behavior in relation to their memorized tutor song (the goal). Spiking patterns of single neurons in both CORE and SHELL subregions during singing correlated with acoustic similarity to tutor syllables, suggesting a process of outcome evaluation. Both CORE and SHELL neurons encoded tutor similarity via either increases or decreases in firing rate, although only SHELL neurons showed a significant association at the population level. Tutor similarity predicted firing rates most strongly during early stages of learning, and SHELL but not CORE neurons showed decreases in response variability across development, suggesting that the activity of SHELL neurons reflects the progression of learning.

S6: Processing the polarization of light

[S6-1] Horsefly polarotaxis is mediated by a segregated ommatidial subtype with spectrally imbalanced photoreceptors, leading to colour-induced polarization artifacts

Dr Gregor Belusic[1], Marko Ilić[1], Martin Wehling[2], Dr Primož Pirih[3], Dr Marko Kreft[1], Dr Andrej Meglič[1]
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Horseflies are arguably the best Dipteran model for the study of polarization vision. We investigated the anatomical and physiological basis for the polarotactic behaviour in the female common horsefly, Tabanus bromius, and tested the findings with behavioural experiments. Using serial block face SEM and intracellular recording in the ventral retina, a random lattice of two ommatidial subtypes was identified: in one type, the UV and UV-green sensitive R7,8, have twisted microvilli and low polarization sensitivity (PS). In the other type, the UV and UV-blue-sensitive R7,8 have straight and orthogonal microvilli and high PS. Thus, horsefly ventral polarization vision is mediated by a stochastically distributed ommatidial type, equivalent to fly 'pale' ommatidia. The findings were tested in the field, by measuring the spectral dependence of horsefly attraction to shiny black balls, suspended below colour filters. Polarotaxis was abolished with a 450 nm longpass filter, showing the necessity of UV and UV-blue-sensitive receptors. Interestingly, polarotaxis was increased with a 400 nm longpass filter, presumably due to the increased intra-ommatidial opponent signal from UV:UV-blue cells. Finally, a blue bandpass filter (450±50 nm) was isolated as a super-stimulus. Here, the filter has increased the UV:UV-blue opponency and suppressed the cross-ommatidial inhibition by the UV:UV-green cells. Our results reveal the substrate for horsefly polarotaxis, mediated by multiple inputs from different ommatidia. The PS photoreceptors are spectrally imbalanced and thus confuse monochrome blue light with polarized light. The findings can explain the attraction of biting flies towards blue objects.

[S6-2] Integration of Celestial and Wind information in the Dung Beetle's Compass

Dr Basil El Jundi[1]
[1]University Of Wuerzburg / Biocenter, Wuerzburg, Germany

Some dung beetles have developed a unique orientation behavior to avoid competition for food at the dung pat. They cut off a piece of dung, form it into a ball and roll it away along a straight path. To maintain their direction, these animals rely on multiple celestial cues, including polarized skylight, as reference for orientation. In addition, wind is often present in the beetles' natural habitat and could play a crucial role as orientation reference when skylight cues lack any clear directional information. This raises the question of how dung beetles define the relevance of different cues and how celestial and putatively wind information are integrated in the beetle's compass. To formulate an understanding of the beetles' compass, we tested their orientation behavior and analyzed the brain activity. Our behavioral experiments show that dung beetles rely on the sun rather than polarized light as main orientation cue. Surprisingly, when skylight information becomes more ambiguous to the dung beetles at high sun elevations, they switch to a wind compass as main reference. The relevance of these cues seems to be defined prior to rolling, when a beetle dances on top of a ball and takes a snapshot of at least the celestial scenery. Neurons of a brain region, the central complex, encode polarization and sun azimuth information, and respond to wind stimuli. These neurons are therefore perfectly suited to integrate wind information into the beetle's sky compass. The reliance on multiple cues generates a robust orientation compass that allows the animal to maintain a straight-line at any moment in time.

[S6-3] Characterizing the sensitivity of polarization vision in invertebrates using ERGs

Mr. Jake S Manger[1], A/Prof Julian C Partridge[1], Dr Zahra M Bagheri[1], **Dr. Jan M Hemmi[1]**
[1]University Of Western Australia, Perth, Australia

Many animals detect and utilize stimuli that humans cannot perceive including the polarization of light. Polarization Vision (PV) is often associated with specialized areas of compound eyes and sky compass navigation. However, an increasing number of animals have been shown to use PV throughout their visual field, with PV potentially integrated into a wide range of visual tasks including object identification and flight control. In order to understand the advantages PV brings in terms of information processing and how the ability to see differences in the polarization state of light is integrated with other aspects of vision, we need better tools to rapidly characterize the polarization vision ability of animals. We here provide results from ElectroRetinoGram (ERG) recordings recorded in combination with a novel dynamic polarization stimulator. The recordings were taken using the fiddler crab Uca dampieri and compared with predictions from a simple neural model of polarization processing. We manipulated angle and degree of linear polarization, stimulus contrast, and intensity. The results show that the crabs have two polarization channels with maximum sensitivity to horizontal and vertical e-vector orientation. Responses increased with increasing degree of linear polarisation (DoLP), with contrast, and with light intensity. We compare PV sensitivities (angular, DoLP, intensity) with those obtained from spatial vision ERGs and behavioural experiments where possible. In summary, our recording and stimulation system provides a quick and minimally invasive tool that can be used to characterize the anatomical arrangement of polarization sensitive visual systems and some of their sensitivity thresholds across different regions of the eye.

[S6-4] New directions for studies of how animals use the polarization of light

Professor Nicholas W Roberts[1], Martin J How[1]
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How animals use the polarization of light as a form of visual information can be categorised in two different ways. The first is as a wide field cue, be it for navigation or habitat selection, and the second as visual information that can be under selection, such as object contrast or signalling for communication. The functional requirements for being sensitive to the polarization of light differs considerably between of these two categories. Viewing broad distributions of polarization such as the overhead skylight pattern, require low-pass spatial filtering, often achieved by the low visual acuity of the dorsal rim areas of insect eyes. However, acquiring visual information from visual signals or using polarization sensitivity as a mechanism to improve visual contrast requires greater levels of discrimination between different forms of polarization. In this talk we will highlight some of our recent work investigating how different animals optimise the acquisition of polarization information and how this is done for different tasks. We will discuss the polarization sensitivity of crustaceans and also how fish use the polarization of their surrounding light field as a broad field feeding cue. We will also comment on some of the important considerations for experimenters when undertaking studies that manipulate the polarization of light [1].

1. Foster, J.J., Temple, S.E., How, M.J., Daly, I.M., Sharkey, C.R., Wilby. D. and Roberts N.W. (2018) Polarisation vision: overcoming challenges of working with a property of light we barely see Science of Nature 105: 27 https://doi.org/10.1007/s00114-018-1551-3

Plenary Lecture 4

[PL4] Colour vision, perception and patterns: insights from coral reef fish

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The natural world provides an inspiring palette of colour signals and highlight the importance of colour in many animals' daily lives. Indeed, colour patterns are used to attract mates, avoid detection, compete for resources and warn predators of underlying defenses. However, to investigate the function and evolution of such signals, we must understand how colour signals are processed and perceived by animals. Recent studies of animal colour vision have focused on the identification of physiological mechanisms, including photopigment and photoreceptor spectral sensitivities and neurons coding for opponency mechanisms, and on theoretical models to predict colour discrimination from this information. However, such data and models cannot replace behavioural tests of colour perception. In this talk, I will present recent studies we have conducted using a coral reef fish as a model system. I will first discuss an innovative method inspired by Ishihara colour vision tests to determine colour discrimination thresholds across different areas of colour space. The method uses an oddity from sample method, which can be used to measure discrimination thresholds and the detection of suprathreshold colours, but may also be used to examine a number of questions about visual processing. Second, I will discuss behavioral studies that have investigated the perception of colour patterns used to avoid predation, including warning signals and disruptive coloration. Specifically, I will discuss how predators may only pay attention to part of the signal when learning to avoid aposematic prey. Finally, I will present work investigating whether fish see visual (lightness) illusions in the same way as we do.

S7: Sensory motion strategies

[S7-1] Towards a brain architecture for visual behavioral selection in Drosophila

Dr Gwyneth Card

How do animal nervous systems integrate sensory information to produce adaptive behavior? Through physiological, behavioral and genetic studies of fly responses to natural stimuli, such as a looming predator, we aim to uncover circuit mechanisms by which animals make behavioral decisions.

[S7-2] Sensory systems influence the locomotion strategy of Drosophila

Ms Kristina Corthals1, Naoyuki Fuse2, Bart R. H. Geurten1
1Georg-August-University Goettingen, Goettingen, Germany, 2Tōhoku University, Senai, Japan

Since most insects lack stereoscopic vision, other cues for distance estimation become prevalent, for example the retinal image shift induced by self-motion, called optic flow. Translational movements result in faster movement of close objects across the retina than distant ones, whereas during rotational movement all objects

move with the same speed. Therefore, only translational movements provide distance information1. Insects overcome this problem by utilizing a saccadic movement strategy, consisting of very short and fast rotations separated from translational movements2-4. These findings suggest a correlation between the saccadic movement strategy in insects and the status of the visual system.
Assessing different visual mutations and completely abolishing visual cues by rearing Drosophila in darkness for over 1500 generations (dark-fly line), we could prove that the separation of translation and rotation is influenced by manipulations of the visual system. The abolishment of visual cues lead to an observed locomotion strategy adaptation to newly prevalent senses like mechanosensation.

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[S7-3] Saccadic movement strategies in swimming animals

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Many animals clearly segregate their movements in translations and rotations. Moreover, they keep the rotations short, they perform saccades. This so called saccadic movement strategy has been linked to optic flow perception and the extraction of distance estimation from optic flow as distances can be estimated from translational but not rotational optic flow: By reducing the duration of rotations, the time during which optic flow distance information can be obtained is maximized. Whereas saccadic movement strategies have been described for many walking or flying insects, we herein present the first analysis of the movement pattern of swimming animals, harbor seals (Phoca vitulina) and cuttlefish (Sepia officinalis). The animals were filmed at high speed when moving in their large enclosures freely and/or in feeding context. In a subsequent video analysis, thrust, slip, and yaw were obtained by manually tracing the animal bodies' center of mass. Comparable to terrestrial animals previously examined, seals and cuttlefish also moved saccadically thereby reaching rotational velocities of approximately 350 deg/s. Interestingly, a context-dependent saccadic movement strategy could be documented for cuttlefish: they move saccadically when swimming at slow speed, a movement mediated by their fins; however, they abolish the saccadic movement strategy when moving at high speed propelled by their siphons. In conclusion, harbor seals and cuttlefish invest in a saccadic movement strategy. Most likely they move in this specify way to optimize distance information from optic flow comparable to terrestrial species.

[S7-4] How do snakes stabilize the olfactory world? Investigating compensatory tongue and head movements in snakes.

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In order to probe the environment, snakes regularly protrude and retract their forked tongue to collect airborne and substrate bound chemical compounds, which are then transported to the vomero-nasal organ. For arboreal snakes however, which hunt in trees, directional information delivered by airborne odor plumes is scarce and potentially impaired by passive movements of the animal induced by, e.g., swinging branches or by active locomotion of the snake itself. To analyze how snakes might compensate for such movements, we recorded video sequences of head movements and tongue flicking behavior in the arboreal amazon tree boa (Corallus hortulanus) and the ground dwelling western diamondback rattlesnake (Crotalus atrox). Snakes were placed on a motorized turntable and rotated at a frequency of 0.1 Hz (±45°). We observed counter-rotational head movements produced by relative movements of the s-shaped anterior body, resulting in a precise stabilization of the head. When the snake´s body and head were stable, the tongue protruded forward along the long body axis of the snake. However, when inaccurate head stabilization was provoked by placing the snake on an excentric position, the tongue was horizontally deflected in a range of ±20°. Additionally, we found that when a stationary snaked observed a moving target, its tongue protrusions followed the direction of the moving target. Taken together, this evidence indicates that snakes do not exclusively move the tongue to deliver odorants to the vomero-nasal organ, but may also actively direct the tongue towards an area of interest.

S8: The evolution of sleep and adaptive sleeplessness

[S8-1] Do all animals sleep?

<u>Dr John Lesku[1]</u>
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When did sleep first evolve? Do all animals sleep? Sleep is typically a behavioural shutdown characterized by immobility, decreased responsiveness, rapid reversibility and homeostatic regulation. Such sleep behaviour appears to be phylogenetically widespread across animals. Indeed, every species that has been adequately studied by sleep scientists has been found to sleep, including arthropods, molluscs, a nematode roundworm, platyhelminth flatworm, and even a brainless jellyfish. This grand evolutionary longevity suggests that sleep fulfils a fundamental, and perhaps inescapable, need. That said, of the 36 (or so) animal phyla, sleep has been demonstrated in just six. Thus, although it appears that sleep evolved early in the lineage of animals has since persisted over evolutionary time, it remains unknown whether these large-scale similarities in sleep reflect homology or convergent evolution. Next, can any animals go without sleep? Sleep is outwardly a maladaptive behaviour that leaves an animal vulnerable to attack (at worst) and unable to do anything else (at best). Natural selection might be expected to favour animals that can forgo sleep in pursuit of waking activities when the benefits of wakefulness outweigh the costs of sleep loss. Here we present preliminary data on the activity patterns of a small marsupial mammal. Males increase their activity during the breeding season, presumably to secure mating opportunities, before they die in a synchronous mass-death. In this way, the amount of sleep might reflect a tradeoff with competing (waking) demands.

[S8-2] Sleeping in a society of honey bees

Dr Barrett Klein[1]
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I will share recent evidence related to sleep in western honey bees (Apis mellifera), including (1) the plight of a bee following a sleep-restricted dancer, and (2) what happens in the antennal lobe of a sleeping bee's brain.

(1) Effects of sleep loss and imprecise communication on the honey bee dance floor: Communication and sleep are important for humans and honey bees alike. Despite this, consequences of sleep loss on communication – both signaling and receiving – are virtually unknown in nonhuman animals. Honey bees (Apis mellifera) can famously signal the destination of a food source with a waggle dance, but sleep-deprived dancers perform fewer and less precise dances. We monitored dance followers with respect to a dance's directional precision and whether or not the dancer had been sleep-deprived. Followers switched dances after following an imprecise dance, and exited the nest after following a precise dance. Bees followed fewer iterations of a dance that was less precise, but only if the dancer was sleep-restricted, suggesting cues associated with sleep loss could affect a follower's foraging success.

(2) Imaging the brains of honey bees sensing odors while they sleep: Honey bees, when awake, process odor information in the antennal lobe and in higher brain regions. Odors elicit an odor-specific, combinatorial activation pattern. We measured behavior and neuronal activity in response to odor in honey bees' antennal lobes, using calcium imaging while the honey bees were awake and asleep.

[S8-3] Sleeping with both eyes open: evidence of behavioural sleep in the Port Jackson shark

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All vertebrate animals studied thus far appear to engage in some form of sleep behaviour (immobility, sustained quiescence, an increased arousal threshold, an incurred sleep debt), but there is currently no data on whether cartilaginous fishes sleep. As sharks represent the earliest stage in the evolution of jawed vertebrates, they may hold vital clues to the evolution of sleep. Sharks respire by forcing oxygenated water over their gills by either continuous swimming (ram ventilation) or by active (buccal) pumping, which facilitates periods of inactivity. This study examines the behavioural and physiological indicators of sleep in a species of buccal pumping shark, Australia's Port Jackson shark (Heterodontus portusjacksoni). Under laboratory conditions, juvenile Port Jackson sharks subjected to normal, constant, and varying photoperiods over 72 hours, show evidence of an activity/inactivity circadian rhythm, entrained by an environmental zeitgeber. These animals also exhibit the behavioural criteria of sleep, such as an increased arousal threshold during periods of prolonged inactivity, tested via the presentation of an electrical pulse stimulus. This study is the first evidence of any species of shark engaging in periods of sleep and provides the first insight into whether sharks are able to monitor their environment over a 24 hour cycle. Ongoing research is focusing on the metabolic and neurophysiological indicators of sleep in this species, in addition to examining species that swim continuously to facilitate gas exchange. The study is important in both helping to trace the evolution of sleep in vertebrates, and understanding the basic behaviour of these apex predators.

[S8-4] First Evidence of Sleep in Flight

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Many birds fly non-stop for days to months, but do they sleep in flight and if so how? When necessary, birds on land can sleep with one eye open, a behaviour associated with wakefulness in the opposite cerebral hemisphere and slow-wave sleep (SWS) in the other. Likewise, dolphins can swim during unihemispheric SWS. Thus, it is commonly assumed that birds alternate sleeping with the left or right hemisphere during long flights, permitting them to maintain aerodynamic control and environmental awareness, while obtaining enough daily sleep to maintain attention during wakefulness. We tested these assumptions in great frigatebirds (Fregata minor) flying over the ocean for up to 10 days, by recording GPS coordinates, flight altitude, head movements, and the electroencephalogram (EEG). We found that frigatebirds can sleep in flight for periods lasting up to several minutes, and usually do so at night while circling in rising air currents. Although SWS was more often unihemispheric in flight than on land, 28% was bihemispheric, demonstrating that unihemispheric SWS is not required for aerodynamic control. Instead, a relationship between the direction of flight and opposing interhemispheric asymmetries in EEG slow-wave (0.75–4.5 Hz) and gamma (30–80 Hz) activity, the later involved in visual attention, suggests that frigatebirds use unihemispheric SWS to watch where they are going. Despite being able to sleep on the wing, frigatebirds slept for only 0.7 h/day, 7.4% of the time spent sleeping on land. The low amount of sleep in flight indicates that the ecological demand for attention, even at night, usually exceeds that afforded by sleeping unhemispherically.

S9: Context-dependent modification of vocal behaviour in vertebrates

[S9-1] The μ-Opioid Receptor System Modulates the Motivation to Sing and Acoustic Features of Female-Directed Song in Zebra Finches

Mr Sandeep Kumar[1], Mr Alok Mohapatra[1], Ms Sudha Sharma[1], Ms Utkarsha Singh[1], Mr Ramanathan Narayan[1], MrVasav Arora[1], Dr Niranjan Kambi[1], Mr Atanu Datta[1], Dr Hanuman Sharma[2], Dr Thirumurthy Velpandian[2], Dr Soumya Iyengar[1]
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Song learning and singing in songbirds (zebra finches) are direct outputs of an interconnected set of specialized brain regions known as the song control system (SCS). The SCS is also linked to neural circuits for reward and motivation (the ventral tegmental area, VTA) and is an excellent model system to study the motivational aspects of vocal behaviour. Earlier studies have demonstrated the presence of the endogenous opioid peptides as well as μ-opioid receptors (μ-ORs) in different components of the SCS and reward circuits. Systemically blocking μ-ORs in male zebra finches led to a decrease in singing and changes in the acoustic features of female-directed (FD) songs. Interestingly, levels of enkephalin and the neurotransmitter dopamine (released by the VTA) are known to increase in Area X, the basal ganglia component of the SCS during the production of FD song by adult male zebra finches. In order to specifically examine the effects of opioid modulation in Area X on singing, we used microdialysis to infuse naloxone (a μ-OR antagonist) and analysed neurotransmitters in the dialysate collected during the production of FD song. Surprisingly, blocking μ-ORs in Area X led to a significant increase in FD song, whereas levels of dopamine were similar to those produced in controls. We also found that the spectro-temporal properties of individual syllables were altered, following changes in μ-OR modulation in Area X. Our results demonstrate that opioid modulation in the SCS can affect the motivation to sing as well as the quality of FD song.

[S9-2] Neural mechanisms underlying vocal sensorimotor transformations

Dr. Arkarup Banerjee[1], Dr. Daniel Okobi[1], Dr. Steven Phelps[2], **Dr Michael Long[1]**
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Although vocal interactions are central to our everyday lives, we know little about the processes that enable individuals to flexibly respond to a vocal partner, a process known as 'turn taking'. Here we characterize the neural basis for similar type of social interchange in the singing mouse (S. teguina), a highly vocal neotropical rodent species capable of producing an audible, stereotyped song. S. teguina often coordinate the timing of their vocalizations to interact with conspecifics in a process known as countersinging. In these studies, we perform the first characterization of the circuit mechanisms underlying sensorimotor integration for vocal coordination in a mammalian model system. We first demonstrate that the motor cortex sends short latency projections to subcortical targets involved in producing the song, allowing us to test the hypothesis that cortical sensorimotor dynamics in S. teguina enable flexible coordination between individuals. To better understand this cortically driven-behavior, we further observe the basic algorithms underlying countersinging behavior, including context-specific changes in song production. We examine responses to the playback of specific sensory stimuli designed to test perceptual boundaries and factors leading to vocal responsiveness. We then test the role of motor cortex in vocal communication by examining behavioral deficits that result from either biasing or eliminating activity within the region. Taken together, these experiments reveal general principles concerning the circuit dynamics underlying interindividual communication and introduce a new model system for understanding the circuit basis of developmental disorders, such as autism, that affect these processes.

[S9-3] Precise motor control in vocal behaviour of marmoset monkeys

Dr Steffen Hage[1]
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Monkey vocalizations have been assumed to be largely innate, highly affective, and stereotyped for over 50 years. Recently, this perception has dramatically changed. Current studies including our own have revealed distinct learning mechanisms during vocal development and vocal flexibility allowing monkeys to cognitively control when, where, and what to vocalize. Here, I will give an overview on our recent studies on marmoset monkeys. I will present new data that are indicating that vocalizations of marmoset monkeys do not consist of one discrete call pattern but are built out of many sequentially uttered units, like human speech. Furthermore, I will give insights into recent studies that indicate a potential role of auditory feedback on vocal development in marmoset monkeys. Finally, I will show first data indicating that marmosets, similarly as macaque monkeys, are able to control their vocal output in a goal-directed way to perform a specific task successfully.

[S9-4] Social context-specific vocal communication in Xenopus

Dr Darcy Kelley
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When an ancestral tetrapod leaves the land and vocalizes underwater, how are communication sounds produced and then shaped to maintain essential social information, and how do they diversify during speciation? We have addressed these questions in the species from the aquatic Pipid sub-genus Xenopus in which social communication is dominated by vocal signaling. In X. laevis, vocal signals in both sexes are specific to social context (male/male vocal competition; female receptivity or unreceptivity). Lesions of the central nucleus of the amygdala prevent males from producing socially appropriate vocal responses to conspecific calls. Microstimulation of the CeA in isolated brains initiates sex-specific fictive calling recorded from the laryngeal nerve. Across Xenopus, male advertisement call temporal and spectral features inform species identity. The return to water in ancestral Xenopus was accompanied by a novel mechanism for laryngeal sound production. Sound pulses are initiated by arytenoid disc separation that mechanically excites two structural resonances, the harmonic dyads, intrinsic to the larynx; dyads reflect properties of intra-laryngeal elastic cartilage septa. Both species-specific individual DFs and the clade-specific dyad ratio are intrinsic to the larynx rather than the result of laryngeal muscle modulation by neural circuits. Species-specific spectral features - DF1, DF2 and the DF2/DF1 ratio - instead reflect intrinsic laryngeal tuning resulting from developmental programs shared by descendants of each Xenopus clade's most recent common ancestor. Species specific call temporal features are determined by tuning of neurons within the hindbrain pattern generating circuit whose components include conserved elements of vertebrate respiratory circuits.

Plenary Lecture 5

[PL5] From sensory perception to foraging decision making - the bat's point of view

Professor Yossi Yovel[1]
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Bats are remarkable aviators and amazing navigators. Many bat species nightly commute dozens of kilometers in search of food, and some bat species annually migrate over thousands of kilometers. Studying bats in their natural environment has always been extremely challenging because of their small size (mostly <50 gr) and agile nature. In the past few years, we have developed novel miniature technology to GPS-tag small bats, opening a new window to document their behaviour in the wild. However, the movement of an animal alone is not sufficient for studying its behaviour and its decision processes. We therefore equipped our miniature GPS devices with an ultrasonic microphone, which allows monitoring the sonar and social communication of freely behaving bats. Because echolocating bats rely on sound emission to perceive their environment, on-board recordings enable us to tap into their sensory ‘point of view’ and to monitor fundamental aspects of their behaviour such as attacks on prey and interactions with conspecifics. This intimate description of their behaviour allows us to examine sensory decision making under natural conditions. I will present several projects that examined how bats combine sensory information with social information in order to optimize foraging. I will also present our current effort to include more on-board sensors for studying of bat Neuro-Ecology including acceleration, EEG, physiology and environmental sensors.

The Walter Heiligenberg Lecture

[WHL] Dominant versus subordinate brains: the establishment and consolidation of hierarchy

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Neuroethological model systems give the opportunity of bridging the gap between natural behaviors and the comprehension of their underlying mechanisms. Gymnotus omarorum is a sexually monomorphic weakly electric fish that inhabits the southernmost border of continental distribution of Gymnotiformes in South America. In its natural habitat, G. omarorum holds symmetric territories between males and females across seasons. During breeding, territory size is correlated with circulating steroid hormone levels. Interestingly, in the non-breeding season, when foraging is the only drive, territories only depend on body size and are established independently of circulating steroid hormone levels. As territoriality is mediated by agonistic encounters, the gonadal hormone-independent mechanisms underlying the non-breeding territoriality of G. omarorum can be evaluated by testing its agonistic behavior in laboratory settings. A clear dominant-subordinate status emerges within minutes in intrasexual and intersexual dyadic encounters of non-breeding G. omarorum. Body size in the only predictor of contest outcome. Dominants are highly aggressive even after the contest is resolved, while subordinates display a sequential pattern of submissive electric signaling and retreat. Both females and males are aggressive, and do not differ in fighting ability nor in the value placed on the resource. Although aggression is completely independent of circulating steroids and persists after castration, non-gonadal estrogenic pathways participate in the modulation of this non-breeding aggression. On the other hand, hypothalamic neuropeptides of the vasopressin-oxytocin family (arginine-vasotocin, AVT, and isotocin in teleosts) are known to be key modulators of social behavior, adapting their actions to different contexts. AVT modulation of the establishment of G. omarorum dominant-subordinate status has been explored by pharmacological and cellular techniques. The non-breeding territorial aggression of G. omarorum provides the clearest example of non-overlapping status-dependent effects of AVT among teleosts: while in dominants AVT promotes aggression without affecting their electric displays; in subordinates, AVT induces an increase in the emission of electric submissive displays without affecting their aggression levels. The endogenous release of AVT during the establishment of dominance is supported not only by pharmacological but also by cellular data. A depletion in AVT content is observed in the soma of AVT neurons in the dominants' preoptic area by immunohistochemistry immediately after the establishment of dominance. In a more long-term, after 2 days of dominance consolidation, a distinctive status-dependent brain transcriptomic pattern emerges. Overall, a long trajectory of neuroethological studies, combining field and laboratory approaches in a wild South American species, enabled the emergence of a new model system, which has already contributed novel aspects in the modulation of aggression in vertebrates.

Plenary Lecture 6

[PL6] Organization of the auditory system in fruit flies

Professor Azusa Kamikouchi[1]
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How does the brain process acoustic information? Revealing the anatomic and functional organizations of the auditory system is indispensable to answer this question. The fruit fly is ideally suited for tackling such tasks, due to its small brain size and a rich repertoire of genetic tools. Moreover, they use acoustic signals to communicate with each other. How does a tiny fly brain evaluate the species-specific communication sound? Toward comprehensive identification of auditory neural circuits in the fly brain, we systematically identified the auditory sensory neurons and their downstream neurons. The anatomic and physiological analyses revealed frequency segregation at the first layer of the auditory pathway and the convergence of frequency information in the subsequent downstream pathways. Second-order auditory neurons have intensive binaural interactions, raising the possibility that the fly is capable of comparing acoustic signals detected at the left and right ears. Based on our analysis, we established the first comprehensive map of primary and secondary auditory neurons in the fly brain, which are characterized by frequency segregation and convergence, binaural interaction, and multimodal pathways. We used this anatomic information to understand how each type of neurons and neural circuits contribute to the courtship-song detection in flies. Activity imaging and silencing of each neuronal type revealed how the selective response to species-specific song was established. These results provide new insights into the neural-circuit basis to adjust neuronal and behavioral responses to a species-specific communication sound.

S10: Vision and memory in crustaceans – neural basis, function and phylogeny

[S10-1] Insights from a formidable companion, the crab Neohelice granulata

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Neohelice granulata is a highly visual semi-terrestrial crab that displays a rich collection of behaviors, including a variety of escape responses, attacks to smaller crabs and diverse conspecific interactions. Using a combination of techniques such as lab and field behavioral analyses, intracellular recordings, calcium imaging techniques and histology we are advancing our understanding of the neural bases of such behaviors. So far, we have focused our research in exploring giant tangential neurons from the lobula (LG) the activity of which greatly correlates with different attributes of an escape response elicited by a visual danger stimuli and with the behavioral changes induced by learning. Interestingly, two classes of LG, MLG1 and MLG2 respond to approaching stimuli with a profile of response that closely follow the dynamic of expansion of the looming stimuli. MLG1 is part of an ensemble of 16 elements distributed uniformly over the whole lateromedial lobula axis. These neurons have large principal neurites that are easily identifiable in unstained brains. I will show new ultraestructural data that illustrate the connectivity patterns of MLG1. I will also present data from behavioral experiments aimed at exploring optomotor responses in two crab species belonging to two different families (Neohelice granulata and Uca uruguayensis), which shade light on the underlying circuit that commands this behavior. In addition, I will talk about other nervous structures identified in the optic lobes, the function and morphology of which are just starting to become clear.

[S10-2] The evolution of crustacean brains and sensory specializations in the midwater hyperiid amphipods

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Studying the nervous systems of animals with highly specialized sensory or behavioral systems often provides insight into how a brain has evolved in response to the animal's natural surroundings. However, correlations between the modifications of certain brain regions and an animal's ecology are mostly suggestive, due to a lack of intermediate forms among closely related species. Here we present a group of crustaceans that provides an excellent opportunity to study how brains adapt when light limitations characteristic of mesopelagic habitats have driven the evolution of a diverse morphology of eyes. Hyperiidea is a suborder of amphipod crustaceans that are abundant members of zooplankton at mesopelagic depths (200-1000 m), where sunlight is reduced to increasingly dim and down-welling blue light. At least 10 different eye types are found, ranging from no eyes, tiny simple eyes, to several forms of complex compound eyes. We show that photoreceptor axons project retinotopically to the lamina, whose axons, in turn, project to the medulla, through an optic chiasm. Some species have a small lobula plate, which receives uncrossed axons from the medulla. One exceptional taxon, Paraphronima, has a true lobula, which receives axonal supply from the medulla through a second optic chiasm. Interestingly, Paraphronima possesses three additional optic neuropils after the lobula, an arrangement that has never been described. While the neural organization of each hyperiid reflects the result of sensory adaptation in its own surroundings, mapping all the neural and habitat characteristics onto the hyperiid phylogeny enables us to reconstruct the evolutionary history of sensory adaptations in this closely related but highly diverse group.

[S10-3] Mushroom Bodies and Mantis Shrimp: Insect-like Brain Structures in a Crustacean

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The exact origin of insects within Crustacea has remained enigmatic, in part due to puzzling discrepancies between neuro-morphological and molecular-based phylogenies of Pancrustacea (Crustacea + Hexapoda). While insect brains are morphologically most similar to malacostracan crustaceans such as shrimp and lobsters, molecular phylogenetics have resolved Remipedia as the sister group to Hexapoda. Remipedes are blind, cave dwelling crustaceans lacking several neural characters that define insect brains such as optic neuropils and lobed sensory integration structures called mushroom bodies. Did insect mushroom bodies and malacostracan sensory integration centers called hemiellipsoid bodies evolve independently to mediate learning and memory or did they evolve divergently from an ancestral structure which was secondarily lost in remipedes? Recent histological investigation of mantis shrimp brains revealed the first example of lobed mushroom bodies in a malacostracan crustacean. At each level of scale from structure to circuits, cell morphology and protein expression, insect mushroom body characters are present in the mantis shrimp. Furthermore, detailed analysis of the mantis shrimp brain resolved a central complex with characters previously only described in insects, including discrete modular organization and paired nodulii. These detailed correspondences suggest that crucial circuitry was conserved over hundreds of millions of years in malacostracan and insect lineages but lost or reduced in other lineages. Alternatively, they are intriguingly complex examples of convergence, perhaps due to evolutionary constrains on learning and memory or action-selection systems.

[S10-4] Divergent evolution of memory centers in malacostracan crustaceans

Dr Nicholas Strausfeld[1]
[1]University of Arizona, Tucson, United States

Despite claims that higher olfactory centers are apomorphies of hexapods and crustaceans, recent studies have challenged this. The discovery of phenotypic homologues of insect mushroom bodies in stomatopod crustaceans confirms that mushroom bodies are indeed present in Malacostraca but have undergone elaborate divergent evolution compared with mushroom bodies of Hexapoda. In addition to Stomatopoda, several other eumalacostracan taxa possess higher olfactory centers manifesting characters that define mushroom bodies: a distal neuropil, the cap, from which extend orthogonal circuits organized as discrete columns expressing proteins required (in Drosophila) for learning and memory. Cap-like distal neuropils have undergone extravagant divergence, obtaining greatest morphological complexity in those eumalacostracan lineages possessing mushroom body-like centers. These include two groups of cleaner shrimps (Stenopididea, Thoridae), pistol shrimps (Alpheoidea), and land hermit crabs (Anomura). In contrast, phyletically more recent Eumalacostraca demonstrates loss of mushroom body characters, except for the cap which is often simplified and assumes the classical identity of a “hemiellipsoid body.” What behaviors unite malacostracans that possess mushroom bodies? Published studies suggest it is the ability to memorize exact locations visited repeatedly. Stomatopoda are ambush predators with excellent memory of place; cleaner shrimps visit specific locations frequented by fish they clean; Alpheoidea are the only crustaceans to have evolved eusociality, for which memory of place and rank is required; land hermit crabs are renowned for memory of sites at which they socially interact. The inference is that, as in the phyletically distant Hexapoda, eumalacostracan mushroom bodies support memory of place and its value, suggesting an ancestral property of these centers.

S11: Facets of brain mechanisms underlying spatial orientation

[S11-1] Neuroethology of phonotactic orientation in field crickets

Dr Stefan Schöneich[1]
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Phonotactic mate localisation requires directional hearing and auditory recognition of the mating call. On an open-loop trackball system, female field crickets reliably steered towards the source of male calling song, even at speaker deviations of less than 2° azimuth. Measuring the tympanic membrane oscillations of the front leg ears with a laser vibrometer revealed from 0° to 30° sound incidence a linear increasing function of interaural amplitude differences with a slope of 0.4 dB/°. Auditory nerve recordings closely reflected these bilateral differences in afferent response latency and intensity providing the physiological basis for a precise auditory orientation that rivals the precision of vertebrate auditory systems. Intracellular recordings in the brains of female crickets revealed how a neural circuit of just five identified neurons forms an auditory feature-detector that recognizes the species-specific sound pulse pattern of the male calling song. This neuronal network receives its auditory input from a single ascending auditory interneuron and the detection of the sound pulse pattern is based on delay-line and coincidence-detection mechanism. An internal delay that matches the pulse period of the calling song is established by a non-spiking brain neuron receiving transient inhibition that then triggers a delayed rebound depolarization. Direct input and delayed responses converge in a coincidence detector neuron, which responds best to the pulse pattern of the species-specific calling song. Although the connecting interneurons are not identified yet, it appears that pattern recognition modulates auditory steering by gating directional information from the ears into the motor pathways for walking.

[S11-2] The role of the Central-Complex in spatial orientation, locomotion, and prey detection

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Complex tasks, like hunting, require high levels of sensorimotor integration. However, before an animal can even begin to forage, it has to transform sensory information into neural codes that represent its position and heading to orient itself in its environment. Only then can its brain start to produce the appropriate commands to move, negotiate obstacles, or find food. For all of these tasks, it relies on information from the same set of sensory organs that have to be evaluated and integrated accordingly within structures of the brain to make decisions on how or where to move and then create the necessary motor output. In the insect brain the central complex (CX) is one target area where these integrations are likely to take place. We performed multi-unit recordings in the CX of cockroaches (Blaberus discoidalis) and praying mantises (Tenodera sinensis) under various experimental paradigms while tracking and categorizing their behavior, to show that the CX is involved in all of these aforementioned tasks. Our results indicate that individual CX cells show head-direction cell-like activity and work as an internal compass. Other cells show movement-predictive properties that are selective for specific behaviors. In our most recent studies on praying mantis, we found cells that show combinations of either reporting movement in specific areas of the visual field, or reporting or predicting self-motion. Lastly, the animal's internal state can alter this motor control. We have found behavioral changes associated with food consumption in praying mantis that can be mimicked by insulin injection.

[S11-3] Acoustic orientation in the dark: About how the brain processes naturalistic echolocation sequences in the fruit-eating bat Carollia perspicillata

Mr M. Jerome Beetz[1,2], Dr. Julio C. Hechavarría[1], Mr Francisco García-Rosales[1], Prof. Dr. Manfred Kössl[1]
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Understanding, how neurons process natural stimuli and control animal behavior is a fundamental goal of neuroethologists. Here we present data on how natural and behaviorally relevant stimuli are processed in the brain of the bat Carollia perspicillata. For short range orientation, bats emit sequences of biosonar calls and listen to echoes. The echoes provide information that enable the bat to orientate acoustically in total darkness. We recorded neuronal signals from the inferior colliculus and auditory cortex, while the bats were stimulated with different echolocation sequences. Stimulating the bat with a sequence carrying echo information from a single object revealed that cortical and collicular suppression sharpens neuronal tuning to specific call-echo pairs of the sequence. Neuronal suppression is stronger in the cortex than in the inferior colliculus. When stimulating the bat with an echolocation sequence carrying echo information from multiple objects, the cortex responds more strongly to echo information from the nearest object. Increasing the stimulus rate with interfering calls from conspecifics does not potentiate neuronal suppression to a level that deteriorates the processing of the echolocation sequence. Altogether, this study provides important information that sheds light on the processing of natural, complex, acoustic streams in the brain.

[S11-4] Vectorial representation of spatial goals in the hippocampus of bats

Ms Ayelet Sarel[1], PhD Arseny Finkelstein[1,2], PhD Liora Las[1], **PhD Nachum Ulanovsky[1]**
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To navigate, animals need to represent their own position and orientation, but also the location of their goal. Neural representations of the animal's position (place cells, grid cells) and orientation (head-direction cells) were extensively studied. However, it was unknown how navigational goals are encoded in the brain. We used a wireless-electrophysiology system to record from hippocampal CA1 neurons of bats flying in complex trajectories towards a spatial goal. We discovered a subpopulation of neurons with angular tuning to the goal-direction (Sarel et al., Science, 2017). Many of these neurons were tuned to an occluded goal – suggesting that the goal-direction representation is memory-based -- a very important property for real-world navigation. We also found cells that encoded distance-to-goal, often in conjunction with goal-direction. The goal-direction and goal-distance signals comprise a vectorial representation of spatial goals – suggesting a novel neuronal mechanism for goal-directed navigation. We propose that this novel vectorial representation in the hippocampus could underlie the ability of many animals and humans to perform vectorial computations while navigating -- including the computation of the "home vector".

S12: What the parts tell us about the whole: Methods and results from automated part tracking

[S12-1] Using machine vision for automated tracking of body and leg positions in freely walking Drosophila

Alice Robie[1], Allen Lee[1], Mayank Kabra[1], Roian Egnor[1], Kristin Branson[1]
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Video-based tracking of animals' body parts allows for detailed behavioral quantification of freely moving animals with high-throughout. It is increasingly easy to collect large high-quality video datasets, but such datasets require the concomitant development of automated tools for detailed behavioral analysis. Machine learning algorithms are rapidly being developed by computer scientists for tracking body parts of humans, but are generally not accessible for use by biologists in animal behavior quantification. To bridge this gap, we've developed software to allow non-specialists to build part trackers of behaving animals using machine vision algorithms. This software has a labeling frontend GUI, and a modular backend for implementing different part-tracking algorithms. It allows the user to easily label 'landmark' points on an animal in order to create a labeled training dataset (including multiple camera views), and then train a tracker that automatically labels these landmarks in unlabeled videos. Using a backend algorithm called Cascaded Pose Regression, we built a tracker that identified position of seventeen landmarks on the body (7 points) and legs (10 points) of Drosophila melanogaster. Videos of these freely walking fruit flies were recorded by a single overhead camera at 150 fps and a spatial resolution of 20 pixel/mm. The current tracker performs with an average cross-validation error rate of less than a pixel based on a training dataset of 3700 labeled flies. The software is general and has also been used to track mice head/tail positions, and insect antennae.

[S12-2] Motion Capture for animal behaviours

Mr Huai-Ti Lin
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Animal behaviours consist of many gestural nuances that are critical to sensory processing as well as motor planning. Characterizing these details is crucial for putting sensory neuroscience in contexts and constructing realistic ethological hypotheses. Motion capture offers one solution to such measurement challenge. The core of a conventional motion capture system consists of a group of cameras each equipped with basic image processing capabilities to segment and recognize specific markers. By engineering the markers' optical properties along with the camera's onboard image processing, a motion capture system gathers marker pixel locations from all cameras and reconstruct real-world three-dimensional positions of each markers. Due to the flexibility of the markers, one can capture precise kinematics of animal parts during behaviour. Over the last 10 years, I have adapted a few different motion capture systems to study the dynamic morphing of caterpillar-inspired soft robots, obstacle negotiation in flying birds, and dragonfly prey interception strategies. None of these systems were subject to the original design of the motion capture systems. Yet through different degrees of customization I was able to capture key behavioural parameters with adequate accuracy. In this talk, I will discuss the use of motion capture in studying animal behaviours and what type of insights we gain through such part-based tracking.

[S12-3] High-speed surface reconstruction of flying birds using structured light

Mr Marc Deetjen[1], Dr David Lentink[1]
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Birds fly effectively through complex windy environments, and in order to understand the behavioral strategies that enable them to do so, we need to quantify the shape and movement of their wings. Wing shape changes dramatically during the stroke cycle, and previous studies show that even small perturbations in wing shape have dramatic aerodynamic effects. However, quantifying wing morphing at high temporal and spatial resolutions is challenging. Here, we present a custom 3D surface mapping method that uses a high-speed camera to view an optimized grid of stripes projected onto a flying bird. By matching the stripes seen in each camera frame with the projected stripes, we can triangulate the 3D wing surface along the grid lines so that we can capture rapidly moving objects at any frame rate with sufficient lighting. In addition, the method is automated, non-invasive, and capable of measuring a shape volume by simultaneously reconstructing from multiple views. For example, using this technique to reconstruct the 3D shape of a flying parrotlet (Forpus coelestis), we extracted wing shape, velocity, and angle of attack and then analyzed key dynamic parameters such as lift and drag. While this novel system is designed to quantify bird wing shape and motion, it is adaptable for tracking the behavior of other quickly deforming animals, especially those which are difficult to reconstruct using other 3D tracking methods.

[S12-4] Comparing Centralization of Locomotor Control in Biological, Computational, and Robotic Models

Izaak Neveln[1], Amoolya Tirumalai[1], Philip Aden[1], Kyle Heiss[1], Simon Sponberg[1]
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Centralization versus decentralization is hypothesized to be an important component of locomotor control. In a highly centralized system, control has a higher ratio of global to local influences, which may be necessary to stabilize low dimensional, global dynamics. However, uncorrelated perturbations might benefit from a decentralized control architecture. Previously, we found that cockroaches running over flat terrain are centralized when we measure mutual information between a control signal with a global output state compared to the mutual information between that control signal and a local output state. We ground this result in a computational coupled oscillator system that has been used to model legged locomotion of running cockroaches. We find that the centralization of a strongly coupled model matches the centralization of the cockroach according to our information theoretic measure. Our centralization measure has the benefit of being model-free, allowing us to apply it to a variety of experimental systems and conditions. For example, we can use this measure to compare centralization of control in cockroaches over a variety of experimental conditions such as running speed and terrain variability. Furthermore, we can compare our measure across systems including robots where the control is specified but overall coupling, which can include complex mechanical interactions, may be difficult to predict. We show that mechanical coupling of a terrestrial robot is reflected in our centralization measure. Experiments on decentralized versus centralized robotic platforms can then be used to generate new hypotheses about biological control.

Plenary Lecture 7

[PL7] Straighten up and fly right: Using a modern fly to reconstruct behaviors of an ancient world

Professor Michael Dickinson[1]
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Over 400 million years ago, a group of tiny six-legged creatures evolved the ability to fly—an event that fundamentally transformed our planet. Equipped with the ability to fly, insects underwent an extraordinary radiation and have dominated every terrestrial ecosystem ever since. In order to fly effectively, these ancient insects must have possessed the rudimentary ability to take off, fly stably, disperse, forage, and land — a core set of behavioral modules that I term 'The Devonian Toolkit'. The fact that the basic architecture of the nervous system is remarkably uniform across species further suggests that many behaviors of modern insects are deeply rooted in this common evolutionary history. My lab is attempting to reconstruct the behavior and ecology of ancestral insects through investigations of the common fruit fly, Drosophila melanogaster. Most experiments on fly behavior and physiology have been confined to small laboratory chambers, yet the natural history of these animals involves dispersal that takes place on a much larger spatial scale. New release-and-recapture experiments in the Mojave Desert confirm that flies can navigate over 10 kilometers of open landscape in just a few hours. Such excursions are only possible because flies can actively maintain a constant heading using a variety of sensory cues. In this talk, I will discuss a hierarchy of neural mechanisms that enable flies to maintain a stable course in the face of external and internal perturbations. Collectively, this new research provides insight into ancient sensory-motor modules that have helped make insects the most successful group of animals in the history of life.

Oral Session 4

[OR25] Representation of 3D space in the entorhinal cortex of flying bats

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Grid cells recorded from animals exploring 2D planes, fire in a hexagonal pattern across the environment. However, many animals navigate through 3D space – but to date, no studies have characterized the 3D volumetric firing of grid cells. Here, we trained Egyptian fruit bats (Rousettus aegyptiacus) to fly in a large room, while we wirelessly recorded single-neuron activity in the medial entorhinal cortex (MEC). Our results revealed structured firing in the 3D firing-rate maps, with multiple firing-fields. The spacing between firing-fields was more variable than in perfect synthetic 3D lattices, but was less variable than shuffled data. Thus, some neurons exhibited a fixed distance scale, without forming a global lattice – supporting a distance-coding function for grid cells. We also found a number of other 3D spatial cell types in the MEC, including (i) 3D border cells, (ii) 3D head-direction cells, and (iii) a new subset of MEC neurons that fired near landing-locations – oftentimes only at very specific preferred-locations. Taken together, these data suggest a rich 3D spatial representation in the MEC of flying bats – including coding of 3D space by grid cells, coding of 3D geometry by border cells, as well as object-related coding in the bat MEC. This neural representation of 3D space could support 3D behaviors such as 3D orientation and 3D navigation in bats.

[OR26] Sound production in decapod crustaceans: behavioral contexts and a newly found role for the circuits of the stomatogastric nervous system.

Dr Marie Goeritz[1], Ashley Flood[1], Dr Craig Radford[1]
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We show that decapod crustaceans are able to produce a variety of sounds. These sounds are associated with a wide range of different behavioral contexts. We show a group of sounds that are specifically related to male competition during mating behavior. Another set of sounds, found in both sexes, coincides with excitation and anticipation of feeding. Interestingly, only a subset of the observed sounds is produced by well-described mechanisms such as leg/claw stridulation. Other sounds are internally produced, without any observable movement of appendices. By simultaneously recording muscle or nerve activity in freely behaving crabs, we show strong evidence that at least one of the underlying mechanisms for these sounds is the movement of the gastric teeth inside the crustacean stomach. This points towards a new and exciting role for the circuits of the stomatogastric nervous system. The need to control stomach teeth movement, not only in the presence of food, but in a variety of different behavioral contexts, might explain the puzzlingly complex array of neuromodulation and sensory feedback that has been found in the stomatogastric nervous system.

[OR27] Underwater Hearing, Internally Coupled Ears (ICE), and Sound Localization in Xenopus laevis

Presenter Leo van Hemmen[1], graduate student of presenter Anupam Vedurmudi[2], long-time collaborator Jakob Christensen-Dalsgaard[3]
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Internally coupled ears or ICE is a hearing adaptation found in several non-mammalian amniotes - in fact, the majority of terrestrial vertebrates - where an interaural cavity of some shape acoustically couples the eardrums. Hence the animal perceives the superposition of outside and internal pressure on the two eardrums, resulting in so-called internal time and level differences, iTD and iLD, as opposed to the interaural time and level differences, ITD and ILD, between the external inputs. When the head size is much smaller than the sound's wavelength, through ICE only a small ITD between the inputs suffices to generate an emergent iLD and enhanced iTD between the eardrums. Here we focus on the African clawed frog Xenopus laevis. Although a terrestrial adaptation, its ICE-like system is uniquely adapted to aquatic hearing in order to compensate for the high speed of underwater sound and its far larger wavelengths than in air. The coupling of the rigid, plate-like, eardrums suspended in a cartilaginous ring leads through a narrow air-filled connection of the small middle-ear cavities to significant iLDs at frequencies associated with the underwater mating calls of the male Xenopus (1.7-2.2 kHz). The frequency of maximal iLD is determined by the internal cavity resonance and, hence, the geometry of the cavity as well as by the material properties of the eardrum and the medium, viz., water. Moreover, the lungs function as a Helmholtz resonator attached to the interaural cavity so that the lungs' inflation improves low-frequency (< 1 kHz) hearing.

[OR28] Audio-vocal integration in echolocating bats

Dr Jinhong Luo[1], Dr Cynthia Moss[1]
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Audio-vocal integration, such as the adaptive speech control by humans, forms the foundation for acoustic communication. Bats are a group of highly vocal mammals and are well-known for their remarkable echolocation capability. Here, we show that echolocating bats are a great mammalian model for audio-vocal integration research. Specifically, we show first that echolocating bats rely on audio-vocal feedback, rather than echo feedback, to control the frequency of their echolocation calls in the presence of acoustic jamming signals. This study demonstrated that like birds and humans, echolocating bats use audio-vocal feedback to fine-tune the features of their vocalizations. Secondly, taking advantage of the short echolocation calls of bats, we show that the amplitude control in noise, often referred to as the Lombard effect, is not only determined by the amplitude of the noise, as has been believed for over a century, but also by the duration of the noise. This study revealed that temporal summation is a key auditory process underlying the Lombard effect. Lastly, we show that echolocating bats offer a unique window to study how vocal production control influences auditory processing. Combining neural recording and mathematical modelling, we report that echolocating bats achieve microsecond precision in marking the timing of acoustic events through synchronous neural firing, and the highest temporal precision occurs with acoustic signals of short duration and broad bandwidth.

[OR29] Neuroethology of sound localization in the pallid bat

Dr Khaleel Razak[1], Mr Dustin Brewton[1], Mr. Stephen Brookshire[1]
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This presentation will discuss novel mechanisms of 2D sound localization discovered in the cortex of the pallid bat (Antrozous pallidus). The pallid bat is a gleaning bat. It uses echolocation for obstacle avoidance and listens passively to prey-generated noise for localizing and hunting terrestrial prey. In a manner of speaking, this bat has to solve an analogous problem as a barn owl. We used behavioral, computational and electrophysiological techniques to identify the behavioral accuracy of sound localization in the azimuth and elevation planes. The bat has one of the best horizontal sound localization accuracy even at very lateral locations. Electrophysiological recordings show that the auditory cortex represents locations in a fundamentally different manner than the barn owl (and mammalian) midbrain. In the region selective for prey-generated noise, there are two binaural clusters distinguished by the shape of ILD and azimuth selectivity functions. The peaked cluster contains neurons with peaked azimuth functions, almost all of which prefer azimuths near 0 degrees (midline neurons). The EI cluster (binaurally inhibited) contains neurons with sigmoidal azimuth functions which respond well to contralateral space. The slopes of EI neuron azimuth functions are arranged systematically in the EI cluster in such a manner that the overall extent of activity in this cluster increases systematically as sound moves to more contralateral space. The elevations sensitivity of the EI neurons are related to their frequency tuning in such a manner that tonotopy influences extent of activity in elevation dependent manner. This is the first proposal for how 2D space is represented in auditory cortex of a mammal.

[OR30] Neural preparatory activity and sensory feedback independent introductory vocalizations drive initiation of learned song in the male zebra finch

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The learned song sequence of an adult male zebra finch is a well-studied example of a learned motor sequence. How such learned motor sequences are initiated in the brain remains poorly understood. Song bouts typically begin with a variable number of short introductory vocalizations (IVs). We have previously shown that the timing and acoustic features of these IVs reaches a consistent state each time song is about to start (Rajan and Doupe, Current Biology, 2013), suggesting the possibility that IVs serve as preparatory vocalizations that help the zebra finch brain reach a "ready" state before song initiation. However, what neural and behavioral mechanisms drive the progression of IVs towards the "ready" state remains unclear. Here, we show that the progression of IVs is independent of sensory feedback but depends on the short-term history of vocalization. Further, neural activity in premotor nucleus HVC begins to change hundreds of milliseconds before the first IV and correlates with successful song initiation. These data suggest that internal changes in neural activity followed by sensory feedback independent repetition of a simple vocalization drive initiation of the learned song sequence. Further, the presence of neural and behavioral preparation make the zebra finch an attractive model system to study the initiation of ethologically relevant learned motor behaviors.

[OR31] Social grouping in caterpillars: proximate mechanisms, from vibroacoustics to sociogenomics

Ms Chanchal Yadav[1], Dr. Myron Smith[1], Dr. Jayne Yack[1]
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Sociality is widespread among larval insects, with many species switching from social early instars to solitary late instars. While the benefits of social groups are widely recognized, the proximate mechanisms (e.g. sensory, genetic) that mediate group formation and maintenance are poorly understood. Our study takes a pioneering approach to understanding such mechanisms by testing hypotheses on the roles of vibroacoustics and sociogenomics. Masked birch caterpillars (Drepana arcuata) (Lepidoptera: Drepanoidea), like several other lepidopterans, transition from gregarious early instars to solitary late instars. Our study explores how (1) neonate caterpillars recruit conspecifics to form groups and; (2) differential gene expression underlies a behavioural switch from social early to solitary late instars. Results to date indicate that early instars generate and utilise complex vibratory signals to advertise food and shelter, and to recruit conspecifics. The shift to a solitary lifestyle by late instars coincides with a remarkable shift in the transcriptome. The transcriptome assembled using caterpillars displaying different behavioral states resulted in a total of 231,348 transcripts and 116,079 putative genes, exhibiting 96% completeness (BUSCO) and was further annotated using Trinotate. We identified 3,097 genes (~3% of total genes) expressed differentially between social and solitary caterpillars, and of these, candidate "social" genes were identified. Preliminary RNAi and qPCR results corroborate our transcriptome analysis and suggest a role for the octopamine receptor gene in mediating gregariousness in early instars. This research explores proximate mechanisms mediating social behaviour using Drepana arcuata as a model. This research confirms the role of vibration-mediated recruitment in social caterpillars and reveals sociogenomic basis to social grouping in caterpillars.

[0R32] Net-caster neurophysiology: Far-field hearing in a nocturnal spider

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Millions of years of evolution can claim responsibility for the origins, modifications, and maintenance of a diverse array of effective and efficient sensory systems. Understanding the design and associated function of sensory systems can provide a foundation for unimaginable technological innovation. For decades, neurophysiological studies utilizing various animal taxa have advanced our understanding of how sensory systems evolve and function. However, while spiders possess a range of unique, independently evolved sensory organs frequently adapted for particular environmental conditions, methodological challenges have hampered our ability to investigate spider neurophysiology. Fortunately, technical breakthroughs have recently opened the door to a neurocomputational approach to studying spider sensory systems. Employing these techniques, we investigate far-field sound sensitivity in a strictly nocturnal predator, the net-casting spider, Deinopis spinosa. Net-casters earn their name through their unique foraging strategy, where individuals suspend themselves above a substrate while outstretching front legs holding a rectangular, fuzzy net. Using this net, spiders actively ensnare prey walking beneath or flying above them. While vision plays an important role in capturing prey off of the ground, the specific mechanisms used to capture flying prey has remained unknown. While recording neural activity in the anterior portion of the D. spinosa protocerebrum, we found spiders to be sensitive to both lower (150Hz) and higher (4400Hz) frequencies. Field playbacks of multiple randomized pure tones resulted in prey strikes for only lower frequency tones. Potential explanations for higher frequency sensitivity will be discussed. In conclusion, we present the first evidence of far-field sound use in a foraging context within a spider.

Oral Session 5

[OR33] Space representation in the goldfish brain

Mr Ehud Vinepinsky[1], Professor Ohad Ben-Shahar[1], Professor Opher Donchin[1], Professor Ronen Segev[1]
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Goldfish, as most animals, engage in some form of navigation in order to survive. Although navigation is a fundamental ability, little is known about the neural representation of space in the fish brain, and specifically goldfish brain. Goldfish have been shown to be able to navigate using allocentric and egocentric cues. Furthermore, there is a defined neuroanatomical region associated with allocentric navigation, the lateral pallium. This region is believed to be homolog to the mammalian hippocampus. Using a novel wireless recording system, we measured the activity of single cells in the lateral pallium while fish swam in a longitudinal aquarium. We found three unique cell types: border cells, velocity cells and speed cells. Border cells are cells which are active when the fish swims near the boundary of the environment. Velocity cells encode the swimming direction and speed, while speed cells encode only the speed independent of direction. Those cells types resemble cell types which are found in the mammalian hippocampal formation and believed to be the building blocks which drive the navigation system. Our study sheds light on how spatial information is encoded in the fish brain and whether the mechanisms of the neural navigation system are preserved across evolution.

[OR34] The Earth's magnetic field and visual landmarks steer migration in a nocturnal moth

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The Australian Bogong moth is a remarkable nocturnal navigator, undertaking a yearly migration over enormous distances from various regions of southeast Australia to the alpine regions of New South Wales and Victoria. After emerging from their pupae in early spring, the adult Bogong moths embark on a long (~ 1000 km) journey towards the Australian Alps, where they seek out the shelter of high ridge-top caves (or rock crevices) to enter a dormant state (aestivation). Towards the end of the summer, the same individuals that arrived months earlier emerge from the caves and begin their long return trip to their place of birth. Once there, moths mate, lay eggs and die. The moths that hatch in the following spring then repeat the migratory cycle afresh. In order to migrate through unknown territories to specific destinations, animals need external cues to perform compass orientation. What external compass cues are used by migrating Bogong moths? By tethering migrating moths in outdoor flight simulators, we found that their flight direction turned predictably when dominant visual landmarks and a natural Earth-strength magnetic field were turned together, but that the moths became disoriented when these cues were set in conflict. Our results suggest a mechanism whereby moths use their magnetic compass to find their inherited migratory direction (like birds) but use one or more visual landmarks as orientation beacons, apparently rechecking landmark fidelity with the compass every few minutes.

[OR35] Magnetic map navigation requires input from the trigeminal nerve in a migratory songbird

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Nature and the sensory basis of navigational map in migratory birds are a matter of hot debate. Behavioural evidence indicates that some migratory songbirds can derive positional (map) information from geomagnetic cues. In previous studies, we could show that Eurasian reed warblers (Acrocephalus scirpaceus) were able to compensate for a 1000 km eastward geographical displacement by switching their orientation direction towards NW in order to reach their breeding grounds. Virtual magnetic displacements showed that magnetic field information alone was sufficient to induce the same reorientation response. Reorientation behaviour after physical displacements required input from the ophthalmic branches of the trigeminal nerves (V1). Neurobiological studies strongly indicate that V1 transmits magnetic information from currently unknown magnetoreceptors to the brain. To test whether V1 indeed carries magnetic map information, we tested Eurasian reed warblers in Kaliningrad region, Russia, under local magnetic field conditions with full access to all environmental cues. The birds showed a significant directional preference towards NE. Birds were split into two groups, one of which underwent bilateral sectioning of V1, whereas in the second one V1 was left intact. Birds were virtually displaced by constantly keeping and retesting them inside a Merritt 4-coil system simulating a 1000 km eastward displacement. Whereas birds with intact V1 compensated their migratory orientation towards NW,

the V1-sectioned birds continued to orient towards NE as before the magnetic displacement. These results clearly indicate that V1 carries magnetic map information for global positioning in Eurasian reed warblers.
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[OR36] Spatial navigation in amphibians: Hippocampal encoding of space based on conspecific vocalizations

Miss Maria Ines Sotelo[1,2], Dr Verner Peter Bingman[3], Dr Ruben Nestor Muzio[1,2]
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How do amphibians, and toads specifically, navigate? How do they find water and breeding ponds? To address these questions in a controlled laboratory setting, we trained eight naïve, partially dehydrated toads (Rhinella arenarum) to look for a water reward in a plus maze. A recorded, con-specific male call was the only cue present to signal the location of the goal (either on the rewarded arm of the maze or in the opposite arm). Animals were able to learn the task in six days and subsequent test trials showed that they were using the con-specific call to locate the goal. C-Fos analysis of brain activity following the last experimental trials showed that the posterior zone of the hippocampal formation of the trained toads had more Fos+ labelled neurons compared to controls. Our results support the hypothesis that sound can be used by Rhinella arenarum to locate a goal, such as a breeding pond, and the hippocampus is involved at some level in the expression of this learned spatial behavior.

[OR37] Sensorimotor strategies for robust flight control under large mass changes in the hawk moth, Manduca sexta

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A hallmark of animal locomotion is robustness of behavior in the face of change. Motor programs must be flexible to contend with injury, adapt to changing environments, and deal with changing body mass (e.g. growth, feeding, gravidity, or load-bearing). To identify strategies for robustness, we take a system identification approach. We can frequently capture shifts in sensorimotor processing with just one or a few parameters showing that neuromechanical systems can manifest simple dynamics, even if the implementation is potentially complex. Here, we focus on dynamic flower handling in hawk moths. In this behavior, moths hover in mid air, cast back and forth up to 14 times a second to track flower movement, and do so in exceptionally dim light. Moths can maintain this behavior even when increasing body mass by >50% during a single feeding bout. Using robotic flowers and control theoretic tools we show that the moths do shift their dynamics when mass increases. However, this change cannot be explained by a simple change in the inertia. The animal's sensory process is adjusting to compensate for the increased mass. Using models of the flight mechanics we show that the seeming complex, frequency dependent change in behavior is actually due to a simple 70% increase in sensorimotor gain at all frequencies. This feedback system identification approach generates descriptions of sensory feedback strategies analogous to the template mechanics models ubiquitous in locomotion –low dimensional targets that are realized, frequently in multiple different ways, in the full complexity of neural systems.

[OR38] Influence of wide-field motion on the signaling of sky-compass cues in the locust central complex

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Many animals rely on visual cues to navigate through their environment. In the desert landmarks are rare but the sky provides several cues exploitable for spatial orientation. One of these cues is the sky-polarization pattern, providing reference for the position of the sun. Desert locusts are able to detect the plane of polarized light (E-vectors) with specialized regions of their compound eyes, potentially enabling them to maintain a steady course on long range migrations. Polarized light signals are processed in several structures of the locust brain, most prominently the central complex (CX), a group of midline-spanning neuropils. Several types of CX neuron hold a topographic representation of zenithal E-vectors, suggesting that the CX acts as an internal sky compass (Heinze and Homberg, 2007). Because optic flow is a prominent visual cue during flight, we asked whether wide-field motion affects the responses of compass neurons to polarized light. In all neurons sensitive to both cues, the presentation of optic flow enhanced the response to the preferred E-vector (i.e. the E-vector eliciting strongest firing), whereas the response to the anti-preferred E-vector remained unaffected. This suggests context-dependent gain modulation in polarization signaling. One type of compass neuron was not responsive to translational optic flow, but to motion simulating body rotation around the yaw axis. Depending on rotation direction this type of neuron was strongly excited or inhibited. As proposed for Drosophila, this type of neuron may be involved in shifting the E-vector preference in the compass during horizontal rotation of the animal.

[OR39] Suppression of echolocation in groups of tri-colored bats

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How bats mitigate mutual interference is a longstanding question that has both ecological and technological implications as biosonar systems continue to outperform man-made sonar systems in noisy, cluttered environments. Echolocating free-tailed bats display a mutual suppression response, slowing their pulse emission rates when flying in groups to gain a net improvement in sonar performance. We hypothesized that mutual suppression is an adaptation for bats that roost and swarm in high densities. To test this hypothesis, we looked for the behavior in the tri-colored bat, Perimyotis subflavus, that is mostly solitary and roosts alone, predicting that it would not decrease pulse emission rates in the presence of echolocation from other bats. We recorded the echolocation of tri-colored bats and measured their emission rates as they flew through an open and cluttered flight room with and without artificial playback mimicking the calls of other bats. The results disproved out hypothesis, showing the same suppression of pulse emission rates with tri-colored bats as we did with free-tails. Instead, mutual suppression appears to be a common mechanism for mitigating interference in laryngeal-echolocating bats. In addition, we recorded emission rates as the echolocation of the bats triggered an artificial stimulus and found that there was a decreased emission rate during the stimulus compared to the rate immediately before the stimulus was played. These findings indicate that a similar mechanism for mutual suppression of echolocation exists among different bat species and may be a used by all bats to cope with interference from conspecifics.

[OR40] Neural control of dynamic 3-dimensional skin papillae for cuttlefish camouflage

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The color and pattern changing abilities of octopus, squid and cuttlefish via chromatophore neuro-muscular organs are unparalleled. Cuttlefish and octopuses also have a unique muscular hydrostat system in their skin. When expressed, dermal bumps called papillae disrupt body shape and imitate the fine texture of surrounding objects, yet the control system is unknown. Here we report for papillae: (i) the motoneurons and the neurotransmitters that control activation and relaxation, (ii) a physiologically fast expression and retraction system, and (iii) a complex of smooth and striated muscles that enables long-term expression of papillae through sustained tension in the absence of neural input. The neural circuits controlling acute shape-shifting skin papillae in cuttlefish show homology to the iridescence circuits in squids. The sustained-tension in papillary muscles for long-term camouflage utilizes muscle heterogeneity, and points toward the existence of a "catch-like" mechanism that would reduce the necessary energy expenditure.

Oral Session 6

[OR41] The “falcon dive” of a killer fly and other sensorimotor challenges of this miniature visual predator

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We have observed that killer flies, miniature dipterans only 4 mm long, employ a ‘diving’ technique to catch prey flying below them. This behavior, where the animal quickly drops vertically head first is reminiscent of a “falcon dive”. To investigate when and why killer flies employ this technique, we have reconstructed the attacks in 3D by recording them with dual high speed video cameras. Unlike falcons, killer flies don’t simply rely on gravity during their dives. Instead, they power their flight, first to achieve higher speeds, and later to slow themselves down. Thus, during a dive, killer flies still “swim through air”, a behavior more alike the aquatic dive of a Guillemot, than the aerial dive of a falcon. On first impression, such dives appear maladaptive because killer flies miss their prey in most dives. However, their high maneuverability allows killer flies to quickly loop around, track their prey and attack again, this time with better aim. Hence, at the very least, a killer fly dive puts the predator closer to the target, and forces the target to turn (and thus slow down) to avoid the attacker, which makes successive attacks possible. Given the importance and prevalence of fast turns in a killer fly attack, we investigated them further with projected targets in a closed-loop arena. Our results show that head movements are necessary for such fast mid-flight rotations and for successful landings, but not for take-off.

[OR42] Nested neuronal oscillators orchestrate motor actions across timescales

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Individual actions rarely occur in isolation; rather, they unfold in specific sequences or in the context of motor programs that can span timescales from milliseconds to hours, serving overarching behavioural goals. How the brain achieves such organised behavioural output across timescales remains poorly understood. We addressed this question in the nematode C. elegans. Using detailed behavioural analysis, whole-nervous-system single-cell resolution functional imaging, and selective neuronal manipulations, we determined the functional relationships between three neuronal oscillators, each acting on different timescales to drive distinct behaviours. On the longest timescale, a brain-wide oscillator commands the forward and reverse locomotion states, as described in our previous work (Kato et al., 2015). Here we describe two types of faster oscillations of the worm's head, each serving different behavioural goals: full-body bends support travelling locomotion, while head-casts allow higher-frequency finer-scale local exploration. Both behaviours occur only during the forward locomotion phase; furthermore, head-casts are superimposed on the full-body bend oscillation also in a phase-dependent manner. Using Ca2+-imaging in both immobilised and freely moving animals as well as neuronal inhibition tools, we identified two classes of neuronal oscillators, one driving full-body bends and the other head-casts. These neurons' activities are phase-coupled to the longer-timescale brain-wide oscillation and, like the behaviours they drive, are nested within the forward motor program. Furthermore, neurons driving head-casts show activity in a similarly phase-nested manner relative to neurons driving full-body bends. These results establish phase-nested oscillations as a repeated dynamical motif of the C. elegans nervous system and suggest a circuit mechanism for orchestrating motor commands across timescales.

[OR43] Descending neuron control of flight behavior in Drosophila

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In most animals, the brain interacts with motor centers in the body through a set of Descending Neurons (DNs) that traverse the neck. We studied the role of DNs on flight control in the fruit fly Drosophila melanogaster. Using cell-type specific driver lines we generated that target individual DNs, we leveraged optogenetics, calcium imaging and electrophysiology to interrogate the function of Flight Descending Neurons (FDNs) innervating the wing and haltere neuropils in the ventral nervous system. We found unique FDNs with bilateral pairs and population FDNs that form small groups of cells with nearly identical arbors. One such population FDNs, DNg02, contained up to fifteen pairs of morphologically identical neurons. By systematically activating subsets of these FDNs using CsChrimson, we observed changes in wingbeat amplitude and frequency during tethered flight. Although the wingbeat amplitude was directly proportional to the number of FDNs activated, frequency changes depended on the frequency level prior to activation up to a specific setpoint. These results, as well as calcium imaging results showing unilateral activity of these cells in response to visual stimuli, suggest the use of population coding for flight control by the FDNs for modulating not only thrust responses during flight but also turning.

[OR44] Ballooning spiders: sensory mechanisms and electric flight

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Some spiders and other wingless arthropods, such as caterpillars and spider mites, disperse aerially over hundreds of kilometres by ballooning. Technically a misnomer, ballooning involves the arthropod releasing strands of silk on which sufficient forces act to provide rapid lift and take off. Air movement and drag forces can generate the lift to make these animals air-borne, however an alternative hypothesis is that electrostatic forces could generate lift. Under ecological conditions spiders and other arthropods will be subject to both air movements and electrostatic fields provided by the atmospheric potential gradient (APG). Here, the ability of spiders to detect and respond behaviourally to electrostatic fields is tested as well as examining putative receptors mechanically. I show that spiders attempt to disperse in response to electrostatic fields, indicating that this could be a meteorological cue for ballooning behaviour. Trichobothria are mechanically displaced by electrostatic fields as low as 100V/m, providing a putative electroreceptor, while also responding to air-flow stimuli. The mechanical response of trichobothria to electric fields and air-flow are distinct, presenting the possibility of discrimination between these two stimuli at the neural level. To date, finding meteorological predictors of spider ballooning behaviour has not provided clear results. APG may be an explanatory factor not only in spider dispersal, but also other ballooning arthropods as well as other species that use passive aerial dispersal mechanisms. Atmospheric electrostatics could provide better predictors of distribution in these species with impacts on agricultural pest management due to the importance of ballooning species both as pest and predators, and also nutrient and pathogen relocation.

[OR45] Timing, Consistency, and Redundancy in a Comprehensive, Spike-Resolved Flight Motor Program

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Neural control of motor behaviors requires the transmission of encoded information to coordinated muscles fulfilling different functional roles. While the role of rate encoding is well-established in motor systems, a growing body of evidence shows that temporal encoding is used to precisely time muscle activity in a variety of motor tasks in a diversity of animals. Though temporal encoding strategies have been examined in single motor units, movement is actuated by many muscles, and little is known about whether information content is tuned to muscle function or how information is encoded across muscles. We recorded spike-resolved EMGs from 10 flight muscles that represent a nearly complete motor program in the hawk moth (Manduca sexta) as it tracked a robotic flower with a simple 1 Hz sinusoidal trajectory in tethered flight, while simultaneously measuring the yaw torque output. We demonstrate that the magnitude of temporally encoded mutual information is higher than the magnitude of rate encoded mutual information for all muscles. Additionally, we find no evidence for differences in encoding strategy between putative flight power and steering muscles. There is a consistent amount of mutual information represented in spike count and spike timing across muscles varying greatly in morphology and function. We also demonstrate that pairwise combinations of muscles encode net redundant information, and that nearly all this net redundant information is encoded temporally. Coordination during complex motor tasks is achieved through temporal encoding, and consistent encoding across functionally different muscles may indicate conserved neural strategies for locomotion.

[OR46] How do flies fly? Modelling the flight of fruitflies in a virtual reality arena

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Through natural selection, insects have evolved into highly adept fliers, where they are able to perform complex manoeuvres during flight even with their grain-sized brain. To investigate the flight dynamics and behaviours of flying insects in response to changes in wind speed and various visual environments, female Queensland fruitflies (Bactrocera tryoni) were tethered and flown in a virtual reality arena. Firstly, the flight dynamics of the flies were determined under open-loop conditions, where they were exposed to sinusoidally oscillating inputs of optic flow (simulated by 4 monitors surrounding the fly) and headwind (generated by a frontal fan), while the forward flight thrust and abdomen pitch angle were recorded. After examining the responses at 4 input modes (wind modulation with constant optic flow and vice versa, simultaneous modulation in phase and counter-phase) over input frequency ranges between 1/32 to 1/4 Hz, the flight controller of the fruitfly was modelled as a second order control system. The pitch response was in phase with either stimulus, while the thrust response was counter-phase (i.e. a maximum response obsereved at minimum stimulus magnitude). After uncovering the flight dynamics, the behaviours during flight were investigated. The flies were initially placed in a forest-like environment with closed loop yaw control, whilst holding a constant forward speed. Early results show an interesting response where the flies seem to avoid collisions with trees, and possibly also inspect them at close quarters, by maintaining a constant distance to the tree - achieved by fixating the tree laterally and keeping a constant visual angle.

[OR47] Backpropagating ectopic action potentials modify information encoding in neurons

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The nervous system must correctly receive, encode, and propagate sensory information for organisms to respond appropriately to their environment. While information propagation in neurons is often seen as unidirectional (i.e. from encoding sites in the dendrites through the axon to the synaptic terminals), neurons are not one-way-streets. Neuromodulators such as monoamines and neuropeptides, for example, can change axonal excitability and induce additional 'ectopic' action potentials in the axon. Ectopic action potentials propagate antidromically towards dendritic regions where they have the potential to modulate the encoding of incoming synaptic or sensory signals. Our data suggests that this is the case, and thus that backpropagating action potentials play an important role in neuronal information encoding. We show that burst parameters corresponding to aspects of sensory encoding by the proprioceptive anterior gastric receptor (AGR) neuron in the stomatogastric nervous system of the crab, Cancer borealis, depend on the firing frequency of ectopic action potentials. Specifically, the sensory burst was delayed, and the spike number and duration of the burst decreased with higher ectopic frequency. Computational modelling predicted that slow hyperpolarizing currents in the periphery facilitate this modulation. We hypothesize that the underlying mechanism driving this modulation is an accumulation of hyperpolarizing currents with higher ectopic firing frequency that modifies fast acting dynamics of voltage-gated Sodium and Potassium channels underlying action potential initiation. For AGR, we hypothesize that slow hyperpolarizing currents are generated by Calcium-activated Potassium channels. Our study elucidates the mechanisms ectopic action potentials act through to modify how information is encoded.

[OR48] The effect of sensory experience on multisensory integration in the weakly electric fish Gnathonemus petersii

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Many species obtain sensory information of the environment through multiple sensory channels. This enables animals to modify their behaviour in response to changing environmental conditions, but also raises the challenge to integrate various and potentially contradictory information. For object detection and analysis, the African weakly electric fish Gnathonemus petersii uses a highly specialized visual sense and an active electric sense. The fish weights these two senses dynamically depending on the reliability of the different modalities during object recognition. To investigate this integration of multiple sensory inputs, we tested whether dynamic weighting is a fixed, innate process or if it is shaped by the training conditions. Fish were trained to discriminate between two metal objects of different shapes or sizes while being able to use both active electrolocation and vision. To modify the sensory experience during training, objects were placed either at a short or at a longer distance from the fish. Subsequently, the discrimination performances of the fish were tested at different distances with either both senses, only vision or only the active electric sense available. Training at short distances led to a dominance of the active electric sense over vision, resulting in an inability or decreased ability of discrimination between objects visually. In contrast, fish which were trained at longer distances were later able to discriminate between the objects with only their electric sense and also by using only vision at various object distances. These results provide evidence that dynamic weighting of multisensory input can be shaped through learning.

Poster Abstracts

Attention & Perception

[P001] Satiety-mediated hunting behaviors are affected by insulin in the praying mantis (Tenodera sinensis)

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The internal state of an animal affects its behavior just as much as the external stimuli that it receives from its environment and, depending on the endogenous neural information that an animal receives, different behavior might result from the same stimuli. Predatory hunting is one such state-dependent behavior that is controlled by the satiety condition of the animal. Here we describe state-dependent changes in hunting strategy through sensory and motor behavioral differences and implicate the neuromodulator insulin as the underlying metabotropic indicator in the praying mantis (Tenodera sinensis). When presented with cockroach prey items, significant decreases in the amount of directed attention, as well as the mean distance and angle of prey localization were observed as the satiety condition increased, indicative of a behavioral paradigm shift. A similar effect was seen in starved mantids injected with 0.05 ml of 200 µg/ml bovine insulin in the abdominal cavity in comparison to a saline control. Furthermore, subgroups of animals were ligated at the neck to prevent hemolymph circulation and were then injected either in the abdomen or directly into the head capsule. These experiments showed that insulin injection into the circulating hemolymph on either side of the ligation had a significant decrease in prey orientation as well as in stepping and striking behaviors, albeit with differing magnitudes of effect. Collectively these results suggest that insulin has effects both in the brain and in thoracic regions such as the thoracic ganglia. The latter case then affects hunting either by altering local control or ascending pathways to the brain.

[P002] The sensorimotor development of naturalistic looking behavior in infants

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Looking is a fundamental component of human behavior, from guiding movements to assessing the social environment. However, there is a great deal that is not known about the development of looking behavior, especially in complicated, free-flowing social interactions. Advances in wearable sensors and computational methods now enable researchers to study the dynamics of looking in three-dimensional environments with precision. Here, we aim to determine how looking behavior changes during the first two years of life, a period of considerable sensorimotor development. We equipped 43 children and their parents with head-mounted eye tracking and wearable motion capture sensors during a session of play lasting up to ten minutes. In this longitudinal study, children were tested at 9, 12, 15, 18, 21, and 24 months of age for a total of 137 sessions, yielding a dataset of 20,125 looks. We first use a data-driven approach to demonstrate that infant visual behavior can be categorized into long and short looks. The proportion of time infants exhibit long, sustained attention to an object decreases as the children age while the proportion of short looks increases. This is consistent with the emergence of the adult phenotype: human adults use quick looks to visually inspect their environment. Further, the emergence of this visual behavior is correlated with the infant's ability to stabilize their head movements, a critical precursor for short looks to occur. Together, these results leverage a novel naturalistic paradigm to elucidate visual attention as part of the infant's developing sensorimotor system.

[P003] Auditory and multimodal localization in generalist birds

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Sensory orientation in space is an essential task for all organisms, and input from all senses is often combined to obtain reliable position information. While much work has unraveled neuronal mechanisms in specialist animals, the generalist layout remains vague. For example, sound localization in azimuth and elevation is largely understood in barn owls, however generalist birds are considered incapable of sound localization in elevation as elevational cues do not appear to be generated. We here present data on auditory localization in a generalist bird species, the chicken. Amplitude discontinuities result from the physics of sound propagation around the head and appear to be used to determine the location of sound in elevation. The animal apparently adopts various head positions to level out the sound source location through tilting movements of the head. The location is laterally to the animal, thus aligning the auditory localization axis with the visual axis in lateral-eyed species. Auditory input reaches the midbrain optic tectum via a previously uncharted relay structure in the lateral reticular formation. The optic tectum receives input from the retina and many other brain structures; the interaction with the isthmic system determines salient points of interest in the sensory environment in a winner-takes-all fashion and thus has a crucial role in bottom-up attention. We recorded auditory spatial receptive fields of neurons in the optic tectum in response to acoustic stimuli presented as HRTF-filtered white noise stimuli in virtual space. On the basis of these data, we will present a hypothesis on auditory and multimodal localization in generalist birds.

[P004] Managing distraction: How male courtship displays attract and retain female visual attention in a jumping spider

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Courtship displays are among nature’s most exuberant expressions of biodiversity. But why are they often so complex? One underexplored possibility is that complex male displays function to manage female distractedness. Female attention is limited, and must often be split between mate assessment and other competing tasks, such as foraging and predator avoidance. Thus male displays may evolve to effectively capture and retain female attention. We investigated this hypothesis in the jumping spider Habronattus pyrrithrix, where male courtship displays involve complex movement sequences, bright colors, and vibrational songs. We used live interaction, video playback, and eyetracking studies to better understand how male courtship traits capture, retain, and manipulate female visual attention. First, we find high levels of female distractedness: 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 effective than locomotory movements at capturing female attention, particularly against complex and/or moving backgrounds. In live interactions, males dynamically modulate their waves: males increase their wave amplitude with increasing distance from females, and 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.

[P005] Bistable visual perception and switching behaviour: Investigating a fundamental brain mechanism with computational biology methods

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In fruit flies, hawkmoths, cats, macaque monkeys and humans, dichoptic presentation of orthogonal gratings induces bistable alternations in perception, i.e., binocular rivalry (BR). In humans, BR rate is under substantial genetic influence ($h^2 = 0.52$). Remarkably, optomotor BR switching behavior in Drosophila engages left-right interhemispheric alternations in LFP activity (Tang & Juusola, 2010). Other examples of such switching activity include alternations between: (i) relative left and right nasal patency (the nasal cycle) in several mammalian species; (ii) left- and right-hemispheric activity during birdsong and sleep; (iii) independent eye movements in the sandlance and chameleon (Pettigrew et al., 1999); and (iv) left and right SCN electrophysiological activity and Per mRNA expression in rodents. Still, examining whether this brain switching mechanism is conserved across taxa has its challenges. We addressed this question by applying computational biology methods to BR rate data from a large sample of healthy adolescent twins (N=1091). A genome-wide association (GWA) analysis revealed no associations at genome-wide significance level (P<5e-8). However, functional annotations and post-GWA analyses revealed a suggestive association between BR rate and CHN1 — an α-chimaerin gene overexpressed in brain tissue. Further research involves GWA meta-analyses to increase power for elucidating BR-associated genes and their relationship with other complex traits (e.g., IQ, language, mental illness). This work will lay the foundation for identifying conserved genes involved in bistable (approach/avoidance) choice behaviour across species and their examination in genetic models (e.g., E. coli, C. elegans, Drosophila, fish, mice), with important implications for understanding the evolution of locomotion, biological rhythms and neuropsychiatric states.

[P006] Mapping of auditory stimulation in the frog brain using Mn-enhanced (MEMRI) and resting state (rsMRI) magnetic resonance imaging

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Acoustic communication is a key feature in the life-history of anuran amphibians. The advertisement call of males serves to attract female mates and to repel male competitors. Anuran amphibians have long been recognized as excellent model organisms in bioacoustics and neurobiology, thanks to their relatively simple brain organization and stereotypic calls. To date, however, most available methods for studying auditory processing in frogs are highly invasive and thus do not allow for longitudinal study designs, nor do they provide a global view of the brain, which substantially limits the questions that can be addressed. The general goal of this study was to evaluate the applicability and suitability of magnetic resonance imaging (MRI) for studying brain activity in frogs, in particular for identifying areas that are responsible for auditory processing. Specifically, we were interested how socially relevant acoustic stimuli (e.g. the species-specific advertisement call) are discerned from less- or non-relevant acoustic signals, such as background noise. We thus designed an experiment, in which we presented three different types of acoustic stimuli (species-specific calls, white noise, or silence) to fully awake Northern Leopard frogs (Rana pipiens) that received a systemic dose of manganese-chloride before the stimulation. After the stimulation, frogs were anaesthetized and transferred to a small-animal MR scanner where manganese-enhanced T1-weighted (MEMRI) and resting-state (rsMRI) scans were conducted. This is the first study that uses MRI technology to study brain function in amphibians. Our experiments show how acoustic information is processed in the amphibian brain, and finally will pave the way for future MRI studies in amphibians.

[P007] Pheromones modulate responsiveness to a noxious stimulus in honey bees

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Pheromones are chemical substances released into the environment by an individual, which trigger stereotyped behaviors and/or physiological processes in individuals of the same species. Yet, a novel hypothesis has suggested that pheromones not only elicit innate responses but also contribute to behavioral plasticity by affecting the subjective evaluation of appetitive or aversive stimuli. To test this hypothesis, we exposed bees to three pheromonal components whose valence was either negative (i.e.,associated with aversive events: isopentyl acetate and 2-heptanone) or positive (i.e., associated with appetitive events: geraniol). We then determined the effect of this exposure on the subjective evaluation of aversive stimuli by quantifying responsiveness to a series of increasing electric shock voltages before and after exposure. Two experiments were conducted varying the time-lapse between shock series (15 min in Experiment 1, and 24 h in Experiment 2). In Experiment 1, we observed a general decrease of shock responsiveness caused by fatigue, due to the short lapse of time between the two series of shocks. This decrease could only be counteracted by isopentyl acetate. The enhancing effect of isopentyl acetate on shock responsiveness was also found in Experiment 2. Conversely, geraniol decreased aversive responsiveness in this experiment; 2-heptanone did not affect aversive responsiveness in any experiment. Overall, our results demonstrate that certain pheromones modulate the salience of aversive stimuli according to their valence. In this way, they would affect the motivation to engage in aversive responses, thus acting as modulators of behavioral plasticity.

[P008] Acute control of the sleep switch in Drosophila reveals a role for gap junctions in regulating behavioral responsiveness.

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Sleep is a dynamic process in most animals, involving distinct stages that probably achieve multiple functions for the brain. Before any sleep functions can be initiated, however, behavioral responsiveness to the outside world must first be turned off. Recent work in Drosophila melanogaster has uncovered a sleep/wake switch in the dorsal fan-shaped body (dFB) of the fly’s central brain, but it is unknown how this switch might govern associated sleep processes, including the acute need to ignore salient stimuli in the environment. While activation of the dFB has been shown to achieve key sleep functions alongside promoting quiescence, recent work has revealed that dFB activation is also associated with synchronized local field potential (LFP) activity in the fly brain, suggesting that electrical communication from the dFB could provide a mechanism to rapidly disengage behavioral responsiveness and thus promote sleep. To address this possibility, we acutely activated the dFB and tested effects on behavioral responsiveness as well as sleep duration, using a paradigm for actively probing awake and sleeping flies with mechanical stimuli. We matched these behavioral experiments with whole-cell patch and LFP electrophysiology to investigate how dFB neurons might be regulating behavioral responsiveness. We found that gap junctions in dFB neurons are required for the sleep switch to modulate behavioral responsiveness as well as LFP activity, suggesting that electrical communication from the dFB links sleep pressure with the acute need to block sensory perception, in order to fall asleep.

Learning & Memory

[P009] Novel operant and classical conditioning paradigms for the cockroach Periplaneta americana

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Classic learning experiments in insects are predominantly conducted in flies and bees[1 2]. Bees express the proboscis extension reflex (PER) which provides an easy means to test a binary conditioned response behavior[2]. They perform well even in complex conditioning tasks[3]. The aim of the present study is to explore the American cockroach as a new model organism for learning and memory taking advantage of a number of its complementary and interesting properties in comparison to other well-established insect models. We developed two novel experimental paradigms for training individual cockroaches. First, we used a classical conditioning paradigm with harnessed cockroaches. We discovered that cockroaches show a Maxillum Labium Extension Response (MaLER) similar to the PER of honeybees and tested different conditioning protocols. The second one is an operant conditioning paradigm based on a T-maze. For this, different forced choice tasks were established. Experiments were conducted by using an aversive (light stimulus) spatial conditioning protocol or an absolute (sugar and salt solution) olfactory conditioning protocol.
Our results demonstrate that P. americana can be successfully trained in classical and operant conditioning tasks and could be used for future learning experiments coupled with neurophysiological recordings.
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[P010] Aminergic neuromodulation of associative visual learning in honey bees

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The honey bee Apis mellifera is a main insect model for the study of visual cognition. Free-flying honeybees learn to associate different visual cues with a sucrose reward and deploy sophisticated cognitive strategies in complex learning sets to obtain such a reward. Despite the intensive characterization of these behavioral capacities, their neural underpinnings remain unexplored as they cannot be accessed in flying insects. Conversely, immobilized bees are accessible to neurobiological investigation but training them to respond appetitively to visual stimuli paired with sucrose reward is difficult. Here we succeeded in coupling visual conditioning in harnessed bees with pharmacological analyses on the role of biogenic amines [octopamine (OA), dopamine (DA) and serotonin (5-HT)] in visual learning. We also studied if and how these biogenic amines modulate sucrose responsiveness and phototaxis as intact reward and visual perception are essential prerequisites for appetitive visual learning. Our results suggest that both octopaminergic and dopaminergic signaling mediate either appetitive sucrose signaling or the associativity between color and sucrose reward in the bee brain. Affecting serotonergic signaling compromised learning performances, probably via an impairment of visual perception. We thus provide a first analysis of the role of aminergic signaling in visual learning and retention in the honey bee and discuss further research trends necessary to understand the neural bases of visual cognition in this insect.

[P011] Color learning by honey bees in virtual reality and in the real world

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To study visual learning in honey bees, we developed a virtual reality (VR) system in which tethered bees walk stationary on a treadmill while they experience visual stimuli with different outcomes projected onto a semicircular screen placed in front of them. The bee movements translate into corresponding modifications of the visual panorama (closed-loop conditions). Using conditioning/transfer experiments between this VR setup and a real Y-maze, we studied whether active vision, enhanced in the real Y-maze, is crucial for learning a simple color discrimination, and whether the learning strategies employed in both contexts are similar. Approximately 55% of the bees trained learned the visual discrimination in both conditions, thus showing that enhanced movement freedom and active vision in the Y-maze did not necessarily improve visual learning. Transfer from the VR setup to the maze did not change the percentage of learners, while transfer from the Y-maze to the VR setup decreased the percentage of learners to 40%. Thus, reducing movement freedom impaired visual performances. We conclude that bees may learn a visual discrimination even under conditions in which active vision was reduced (VR). When the task is learned in a scenario promoting the use of active vision (Y-maze), suppressing movement freedom impairs performance either because of enhanced stress or because their access to active vision is restrained. Our results indicate that bees may use different strategies to learn a simple discrimination in VR and in the real world, and that these strategies differ in their sensitivity to a change in context.

[P012] Conditioned inhibition of the phototactic response in honey bees: a novel protocol for the study of aversive learning and memory

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The honeybee is a well-established invertebrate model for the study of learning and memory. It has the advantage of exhibiting robust learning and memory in immobilized preparations in the laboratory. The conditioning of the proboscis extension response (PER) and of the sting extension response (SER) are two excellent examples of standard, controlled Pavlovian protocols used in bees for the study of appetitive and aversive olfactory learning and memory, respectively. Studies on visual learning and memory have been mostly restricted to an appetitive dimension. Moreover, laboratory protocols allowing the study of visual aversive learning in bees are scarce. Here we introduce a novel aversive conditioning protocol in which freely moving bees confined within an experimental arena learn to associate an attractive spectral light with an electric shock. Our setup consists of a flat arena divided in two halves. The bee is free to explore both compartments under dark conditions (red light). When a trial begins, the compartment in which the bee is located remains dark while the other is illuminated with a spectral color (green or blue) thus triggering a phototactic response. Entering the illuminated side is followed by an electric shock so that bees learn the color-shock association and progressively avoid going to the illuminated side. We show that the learning established in our protocol is robust and leads to the formation of mid-term (protein synthesis independent) and long-term (protein synthesis dependent) memories. We discuss the potential of this novel protocol for studies on aversive visual learning in bees and define future research avenues derived from this learning form.

[P013] Navigation on the ground and on the tree in the nocturnal bull ant, Myrmecia midas

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The night-active bull ant Myrmecia midas faces the challenge of navigating under dim conditions. Foragers start off in the twilight of the evening, heading to a specific tree for some hours of the night, sometimes (~30% of the foraging force) to the tree right where their nest is at. We displaced ants on the ground and found evidence that they use predominantly the surrounding landmark panorama for homing. Ants that forage at their nest tree, however, are not oriented if displaced onto the ground 5m from their tree. We displaced ants onto trees, and found that they also use the surrounding terrestrial panorama for getting to the correct side of the tree according to where their nest is. Blocking the surrounding scene led to disorientation in both cases. Use of the terrestrial visual panorama is a key navigational strategy for Myrmecia midas.

[P014] If you say freeze, I may freeze with you: learning through self-experience determines the meaning of the behavior of others

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Previous work from our lab has shown that rats freeze upon the display of freezing by con-specifics. In addition, this form of observational freezing requires prior experience with foot-shock, suggesting that freezing becomes an alarm cue through learning. We hypothesized that, during exposure to shock, rats associate their own defense responses with aversive stimuli. Consistently, we found that rats exposed to shock that did not experienced freezing do not respond to the display of freezing by their cage-mate. To test if the experience of freezing is sufficient to support observational freezing, we exposed observer rats to stimuli known to trigger freezing innately. We choose 2MT, an odorant derived from fox feces, and looming stimuli, an expanding shadow that simulates a large object on collision course. 2MT effectively induced innate freezing. However, rats showed no sign of fear learning (contextual freezing was absent) or observational freezing. Looming stimuli also triggered robust innate freezing but only very low freezing to the context or during the social interaction. Together our results support the hypothesis that freezing becomes an alarm cue through its association with aversive shock.

To further investigate this question, we paired optogenetically triggered freezing with shock, and found that it resulted in the display of observational freezing. These findings suggest that learning about one's own behavioral responses permits using the behaviors of others as cues about the environment. We are currently investigating how freezing is being represented during learning.

[P015] Gustatory perception and habituation in the moth Agrotis ipsilon: modulation by sex pheromones and biogenic amines

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Insects integrate various environmental information such as the presence of food or potential mates. Some elicit stereotyped responses (e.g. sex pheromone in male moths) whereas other can be integrated through learning. We are especially interested in the way male Agrotis ipsilon moth integrate an exposure to sex pheromone and information about the availability of food, and the underlying neurophysiology. To assess this point, we developed a non-associative gustatory learning, habituation to sucrose. During habituation, the moth stop extending its proboscis in response to antennal sucrose stimulation if it is not fed after these stimulations. For the first time in moths, we carefully described this phenomenon and the parameters affecting it (sucrose sensitivity, season, timing, age. We also showed that at some pre-exposure times, sex pheromones of Agrotis ispsilon but also the related sympatric species Agrotis segetum can modulate it, in spite their leaving intact the sucrose sensitivity. Interestingly, biogenic amines modulate sex pheromone responsiveness, and in other insect species, sucrose sensitivity. Using octopamine and dopamine injections, we found that sucrose sensitivity is modulated by these drugs in moths too; consistently it affects habituation.
This work was supported by grants from ANR (project PheroMod) and Sorbonne Universites (emergence project HAPA).

[P016] Were things different when I left? An experimental and modeling analysis of bumblebees dealing with visual conflict when returning home.

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Foraging bumblebees fly back and forth between their nest and patches of flowers, to collect food to feed the larvae. While flowers are easily recognizable, the entrance of the nest, often placed in a mouse burrow, is not. Therefore, learning the arrangement of conspicuous visual landmarks surrounding the nest is essential. In this project, we analyze how the homing performance of bumblebees is affected if the visual surroundings have changed during a foraging trip. Natural environments often provide some distant visual cues and closer ones (i.e. some trees versus some pinecones around the nest hole). We mimicked this basic environmental feature by a lab set-up where we could alter the environment during the return flight by setting distant and nearby cues into conflict. The wall of a cylindrical flight arena was covered by an asymmetric pattern of three vertical black bars (the background cue), while a set of three small cylinders (the local cue) were placed close to the nest hole. The bumblebees were trained with the two cues placed in a certain spatial arrangement. After observing only straight directed return flights, we recorded flights after setting the cues into conflict by varying their angular relation. We analyzed the consequences of the conflict angle on the bumblebees' strategy to find the home and their search area relative to each cue. Furthermore, we tested how the performance of popular vision-based homing models is, compared to the experimentally analyzed homing performance of bumblebees.

[P017] Representation of large-scale spaces in the hippocampus of flying bats

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Most animals navigate distances spanning from hundreds of meters to many kilometers. However, over forty years, hippocampal research focused on spatial representations in small laboratory environments. Nothing is known about hippocampal neural codes for large spatial scales – the scales of natural navigation of rodents, bats and other mammals. Here we addressed this question for the first time, by developing a unique setup – including a very large environment with ethologically-relevant scale. We studied the Egyptian fruit bat, because these bats are excellent navigators over large scales, and have rodent-like hippocampal spatial representations in small laboratory environments. We developed a wireless neural-logging system, which allows recording single-units over unlimited distances. We built a 200-meter tunnel where bats can fly freely; bat's position was tracked using an RF device – yielding high accuracy of ~10-cm. Bats flew back-and-forth along the tunnel, more than 100 laps per-session (>20-km total distance). Preliminary recordings from hippocampal area CA1 showed some firing properties that were similar to findings in small-scale laboratory environments, such as directionality and field-asymmetry. However, most properties were very different: (i) Individual cells exhibited multiple fields per neuron. (ii) We found very large fields in dorsal CA1 – up to 20-30 meters. (iii) A given neuron could exhibit multiscale spatial coding – with different place-fields of the same neuron having very different sizes, ranging from 1-2 m to 20-30 m. Taken together, the firing properties of CA1 neurons in this large-scale environment suggest a representation that is very different from findings reported in the laboratory.

[P018] Effects of early experience on spatial representation of large-scale environments in the bat hippocampus

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The proper function of brain circuits depends both on processes that require normal experience, and on processes which are independent of experience. The long-term effects of altered sensory experience on the brain have been extensively studied, but very little is known about the long-term physiological effects of abstract, cognitive experiences. The hippocampus offers an excellent substrate for addressing this question, because this high-level brain region is very far removed from the sensory periphery, and it contains abstract representations of space, which are important for navigation. Several studies have examined the normal development of hippocampal spatial representations in rat pups, during ontogeny; however, it is unknown how alterations in early experience affect spatial representation in the adult hippocampus. We are addressing this question by investigating adult spatial representation of a very large naturalistic environment in the hippocampus of laboratory-born bats, which were never exposed to large-scale environments outdoors – and compare it to wild-born adult bats, which were exposed to very large spatial scales. The main difference between these two groups of bats is in a very abstract parameter – the spatial scale of the environment they experienced – whereas the sensory and motor experiences are normal for both groups. We employ a miniature wireless electrophysiology system to record place-cells in hippocampal area CA1 of laboratory-born and wild-caught bats that are flying in a very long 200-m tunnel, performing the same task. Here we will present preliminary neuronal recordings that aim to elucidate how hippocampal representations are shaped by abstract features of early experience.

[P019] The automated flight room: Studying three-dimensional spatial navigation and its underlying neural codes in free-flying bats

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Bats, as the only mammals capable of self-propelled flight, freely navigate in three-dimensional space. They are renowned for their ability to extract information about the environment through their active echolocation system (biosonar), but visual, passive auditory and often olfactory signals are often used for navigation as well. How this multitude of sensory inputs are used for navigation we are only beginning to understand. Since the discovery of position-coding neurons, considerable progress has been made in unraveling the neural mechanisms underlying how the brain guides navigation through complex environments, but much remains unknown, especially with respect to the influence of sensory inputs on ongoing neural activity. We designed a sophisticated setup with which we can record flight behavior, echo-acoustic attention and neural activity in a highly controlled manner. Specifically, the design of our system facilitates fully automated training, thus reducing the great variability of manual training and increasing the number of trainable animals. Here, we describe a task in which bats are trained to approach in flight one of four targets to obtain a food reward, where a correct target is marked by either a visual, auditory or echo-acoustic cue. By varying the intensity, we can reduce the reliability of the sensory cue used for this navigational task and ask how this is reflected in the bat's psychometric navigation performance and ongoing neural activity. This study of navigating bats coupled to cellular-resolution measurements of brain activity during free flight, will provide important insight into how the mammalian brain supports complex three-dimensional navigation.

[P020] Impact of social experience on synaptic density in the mushroom bodies of the paper wasp Polistes fuscatus

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Polistes wasps establish a social hierarchy among nestmates that guides division of labor. In Polistes fuscatus, individuals can recognize nestmates using visual cues provided by facial and abdominal markings. Visual information is processed in the mushroom bodies of the insect brain, which may accordingly contribute to the recognition of individuals via visual cues. Wasps were reared in their normal social environment to preserve their facial recognition ability, or raised in social isolation to reduce this ability. Brain sections of adult wasps were immunolabeled with the synapsin antibody anti-SYNORF1 for imaging on the Zeiss 880 Laser Scanning confocal microscope. The density of the microglomerular synaptic complexes in the lip and collar of the mushroom body were analyzed blind to rearing condition using Imaris analysis software. Statistical analysis of the density of microglomeruli in the collar region of both groups showed no difference between the two rearing conditions. The unchanged synaptic density of the socialized individuals compared with the isolated individuals indicates that synaptic plasticity in the mushroom body neuropils may not be required for facial recognition, or that such plasticity may only be evident earlier in adult life. This study provides a foundation for the use of P. fuscatus for further exploration of the neural mechanisms of learning and memory.

[P021] Long-term activity-dependent elevation in NO concentration mediates LTP expression and maintenance in the octopus vertical lobe

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The octopus vertical lobe (VL) is important for the acquisition of long-term memory and, accordingly, is organized as a fan-out fan-in association/classification network. Previously we showed that the glutamatergic fan-out synaptic connections demonstrate a robust activity-dependent NMDA-independent long-term potentiation (LTP). Recently we found that nitric-oxide (NO) synthase (NOS) inhibitors reversibly block expression and maintenance of this LTP. These findings suggest a very simple and novel 'molecular switch' mechanism in which a single molecule, NO, mediates both presynaptic expression and the maintenance of the very long lasting protein synthesis-independent LTP (>10h) through NO-dependent NOS reactivation. While NADPH-diaphorase histochemistry supports the presence of NOS in the VL, we could not find any indication for the involvement of the common NO-dependent cGMP cascade in LTP. Moreover, NO-donors and NO-scavengers neither facilitated nor inhibited it, respectively. These negative results suggest the involvement of processes functioning at a higher NO concentration than those known to activate the cGMP cascade, for example, a non-enzymatic direct protein s-nitrosylation. Therefore, here we measure NO concentration in the VL neuropil amperometrically. Indeed, induction of LTP is accompanied by long-term increase in the amperometric signal corresponding to the oxidation potential of NO (750 mV). This increase reached the μM range, significantly higher than that found for activation of the cGMP cascade. Altogether these results suggest that independent evolution led to the selection of a simple molecular mechanism in which a single molecule (NO), likely through non-enzymatic processes, mediates both presynaptic LTP expression and its very long-term maintenance.
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[P022] Transgenerational effects of stress on song learning

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The environment animals experience during development can have sustained effects on their morphology, physiology, behavior, and fitness. Sometimes these effects persist to adulthood, or even across generations. The direct effects of developmental stress on song learning are well documented in a range of species, but to date whether vocal learning is also detrimentally affected in subsequent generations remains to be tested. This project is the first to examine whether developmental stress can have transgenerational effects on song memory. In order to test this we used the zebra finch (Taeniopygia guttata), an iconic songbird species, and exposed developing offspring to elevated corticosterone levels during nestling development, or the control vehicle only. Allowing these birds (F1) to develop and breed, we sought to quantify the effect of parental stress on their F2 offspring. Here, I present the data from the initial experimental generation, assessing the physiological effects of developmental stress on F1 birds. Using a combination of quantitative magnetic resonance and condition indices to estimate body composition, our results show for the first time that the effects of early developmental stress on body fat and mass are not only dose-dependent, but persist into adulthood. Furthermore, I present data to test whether these effects persist across generations into the F2 birds. I will also discuss the impact of parental stress on vocal learning, comparing song copying across treatment groups. Together, this work addresses the long-term implications of early life stress, not only for individual physiological condition but also for cognitive functions.

[P023] Influence of developmental stress on songbird memory formation

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Vocal learning allows for complex communication for the benefit of resource defence and securing individual fitness. In birds, vocal learning allows for the production of sexually selected complex song, dialects and song copy matching. But conditions during early development may greatly affect the process of song learning, with early life stressors compromising both song production and complexity, mediated by changes in the neural centres controlling song output. However, to date no studies have tested whether early-life stress affects the mechanisms underlying vocal learning per se (and, in particular, auditory memory formation processes), in contrast to song production. To test this, we experimentally stressed male nestling zebra finches, and in two separate experiments, tested their ability to respond to their tutor song as adults, using either immediate early gene (IEG) expression or electrophysiological response. Once adult, nutritionally restricted nestlings exhibited a reduced response to playback of tutor song, as demonstrated by reduced expression of two IEGs (Arc and ZENK) and also reduced neuronal response, in both the caudomedial nidopallium (NCM) and mesopallium (CMM). Furthermore, nutritionally stressed males also showed impaired 20-hour neuronal memory for song playback in adulthood. These results indicate that the neural response to a learned acoustic stimulus is detrimentally affected by developmental stress. Our study demonstrates for the first time that vocal learning is condition-dependent and highlight the impact early-life experiences can have on learning and memory formation.

[P024] Extending the T-maze to evaluate socially motivated behavioral profiles in adult zebrafish

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Zebrafish are highly social and exhibit complex behaviors whose neural and genetic basis is not well understood. We designed a behavioral paradigm to simultaneously test multiple behavioral characteristics, namely, associative learning, decision making, social motivation, anxiety and short-term memory as well as exploratory tendencies. We used visual social reward for motivating directional turns in freely-swimming adult zebrafish, Danio rerio. Our goal was to minimize handling stress, while allowing animals to continuously learn. To enable this, we extended the T-maze by adding a return arm with a 90° turn at each end of the "T". This required the test subject to make four successive turns to return to its original release location. Gates placed at strategic locations closed behind the fish to facilitate forward swimming. Exploration allowed the fish a glimpse of conspecifics placed within an inner tank. This prompted the test subject to make a second turn and approach conspecifics via the return arm. Finally, preference for a dark environment (safety) lured the test subject to make two more turns before reaching the original release location. For each successive test, recall of social reward (memory) motivated the fish to re-explore the maze and make either right- or left-turn decisions. Three successive reward-driven turns to the rewarded side ended the trial and generated a logistic learning curve. Significant learning occurred within 30 minutes (n=5). In addition, relative-time spent within each section of the maze generated a socially-motivated behavioral profile with anxiety exhibiting the highest variation. Our paradigm can be adapted to study behavioral characteristics in many species.

[P025] Distance estimation in a coral reef fish, Rhinecanthus aculeatus

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The Picasso Triggerfish, Rhinecanthus aculeatus is a territorial coral-reef fish of the Indo-Pacific Ocean. Their territories are large and visually complex and they spend much of their day foraging for small fish. An ability to navigate efficiently is crucial to their survival and success. One mechanism by which they could move between patches and return home is path integration. This involves the animal continuously updating its distance and direction travelled such that it can follow a direct vector home. To understand how the path integration system in these animals works, we must explore how they collect this distance and direction information. Previous studies have looked at directional sensing in fish in the context of a long-range compass, with evidence that different species can use a time compensated sun compass, magnetosensation or polarised light to discern their direction of travel. However, the cognitive and mechanistic basis of distance estimation remains unexplored in fish. Using the Picasso Triggerfish as my model system, I present the results of a naturalistic operant conditioning task providing the first direct evidence that fish can keep track of their linear distance travelled. I also present initial findings from exploring the mechanistic basis of distance estimation in these fish, extending previous research with bees, ants and spiders as well as humans and rats. I test the optic flow, time taken and idiothetic cue hypotheses in the fish odometer. These results begin to bridge the gap between our understanding of the path integration system in fish and terrestrial animals.

[P026] Ducks and Decussation: Long and short term investigations of interhemispheric transfer of visual information in birds

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Eutherian mammals are unique in that sensory input from each eye is exchanged and shared between left and right brain hemispheres through the corpus callosum. All other vertebrates lack this structure and hence interocular information exchange is more restricted, raising issues of how information acquired with each eye contributes to the control of behaviour. We investigated interocular transfer in birds, using local-area homing behavior in pigeons (Columba livia) to investigate long term transfer and filial imprinting in pekin ducklings, Anas platyrhynchos domestica to investigate short term transfer. We present here the results of this program of research, showing the behavioural effects of asymmetric and incomplete brain decussation in the long term visuo-spatial integration across hemispheres of homing information in pigeons. We further show, using monocularly imprinted and tested ducklings, that visual information gathered with one eye is laterally isolated for at least 3 h after exposure, suggesting two exclusive streams and stores of visual information in the avian brain. These results together suggest an avian visual umwelt dramatically different from that of mammals, and may provide one adaptive explanation for such phenomena as avian eye dominance and eye alternation in terms of acquiring the most complete or useful visual information.

[P027] Long term aversive taste memory in Drosophila

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The decision what to eat, or not, is very crucial and can lead to serious illness or death. To avoid such fate, animals evolved a sensitive taste system to avoid potentially dangerous food. And in case they encounter novel food, they can form a memory about it to guide their ingestion behavior the next time. The information that animals learn about food depends on many factors including how beneficial it is; if it causes lethality, illness or just discomfort; or how assessable the food is prior ingestion. Previously, we described Aversive Taste Conditioning (ATC) in Drosophila that leads to a short term memory and we characterized the neuronal architecture underlying this type of learning. In mammals, on the other hand, Conditioned Taste Aversion (CTA), a different form of learning leading to a long term memory is studied almost exclusively. There, a pairing of taste of food with later illness or nausea reduces the willingness of animals to subsequently consume food of that taste. We demonstrate that these two forms of taste memories are fundamentally different. Here, we describe a new taste memory assay in Drosophila that is also long-lasting. We characterize the properties of this assay including the characteristics of unconditioned stimuli, the valence of the conditioned and unconditioned stimuli, the identity and intensity specificity, its persistence and its biological relevance. We then use these assays to study the independent processes leading to different forms of memories within the same modality, and to describe the neuronal architecture and genetic components involved in this type of memory.

[P028] Morphology and function of the hemiellipsoid bodies of the crab Neohelice granulata; their role as high-order memory centers

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Despite unique adaptations that animals have in accordance to their lifestyle and the conditions of the environment in which they live, many of the basic mechanisms that allow them to learn and store acquired information to modify their behavior are conserved across evolution. In this regard, the molecular machinery involved in neural plasticity and the dynamics of the memory processes are common throughout vertebrate and invertebrate species. Theoretically, this occurs because the circuitry and brain organization of the ancestral bilateral animal has already provided an effective solution that allows the acquisition of different kinds of information and the organization of memories as internal representations. One of the iconic neural structures that bear this role in vertebrates is the hippocampus, also referred to as archicortex since it is phylogenetically considered to be the oldest brain region. In invertebrates, the corpora pedunculata or mushroom bodies are supposed to play a similar role as the archicortex of vertebrates. Here, we present functional, neuroanatomical and immunohistochemical data of the hemiellipsoid bodies of the crab Neohelice granulata. This structure has been historically proposed to have a similar function to that of the mushroom bodies of insect. N. granulata shows hemiellipsoid bodies that are similar to those described in other true crabs, and its structure resembles that of calyx-less corpora pedunculata that are observed in several insect species.

[P029] Dopamine release in mushroom bodies of the honey bee (Apis mellifera L.)

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Insects learn rapidly to associate sensory stimuli with punishment or reward, and mushroom bodies (MBs) of the brain play a critical role in the formation of such associations. In the fruit fly, Drosophila melanogaster, aversive (electric shock) stimuli have been shown to activate subpopulations of dopaminergic neurons with terminals in the MBs. While evidence suggests dopamine (DA)-induced synaptic plasticity underpins the formation of aversive memories, the mechanisms involved have yet to be fully resolved. Using the honey bee, Apis mellifera, we have examined the kinetics of DA release and reuptake in the MBs. Using fast scan cyclic voltammetry, we show that DA increases transiently in the MBs in response to electric shocks applied to the abdomen of the bee. The magnitude of release varies both with stimulus duration and with stimulus intensity, and a strong correlation can be identified between DA release and the intensity of behavioural responses to shock. With repeated stimulation, peak DA levels increase. However, the amount of DA released on the first stimulation pulse typically exceeds that evoked by subsequent pulses. Intriguingly, pairing shock with odour presentation enhances DA release. We consider the functional significance of this and the potential engagement of the 3 distinct receptor types that mediate dopamine's effects in MBs of the brain. Our results provide an important step towards understanding the modulatory actions of DA in the MBs of the bee.

[P030] Neuronal correlates of behavioral plasticity in social insect brains - approaching the next level of detail

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Social-insect colonies often comprise thousands of individuals and have been described as “superorganisms”. The workers, as the main individual ‘unit’ for the functioning of the superorganism, show a highly adaptive behavioral repertoire enabling the colony to express emergent responses to varying environmental conditions. Variations in behavior such as the transition from an indoor worker to a forager, or learning and memory processes, have been correlated with neuroplastic changes in the brain. These changes in turn are influenced by age- and task-related processes and may be controlled by internal programs and environmental stimuli. After having taken volumetric analyses of neuropils as a widely used measure for neuroplasticity to the level of synaptic complexes, we now aim to gain a more detailed understanding of changes in synaptic circuits at the subcellular level to obtain insights in cellular and molecular processes mediating behavioral plasticity. We applied and adapted, for the first time, array tomography for the use in the brain of honeybees and ants. This technique combines the advantages of two worlds by correlating images taken from the same ultra-thin resin sections with electron microscopy and super-resolution fluorescence microscopy. The overlay of EM and fluorescent images of ultra-thin serial sectioned tissues combines high-resolution 3D-morphological models of synapses with additional super-resolution 3D-information of synaptic-protein localization. The resulting identification and localization of molecular key components for neuronal plasticity provides an essential pre-requisite to manipulate behavior at the level of plasticity-related genes. Supported by DFG SFB 1047 (B6) to WR.

[P031] Learning walks trigger synaptic plasticity in two visual pathways of Cataglyphis ants

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Cataglyphis desert ants are impressive navigators. The ants use a celestial compass for path integration during long foraging trips, and landmark guidance whenever available. To acquire navigational information prior to foraging, Cataglyphis noda performs stereotyped learning walks comprising small loops around the nest entrance, repeatedly interrupted by body turns with stops and precise gazes back toward the nest entrance. By manipulating celestial cues during naïve learning walks we asked (i) whether the celestial compass serves as directional reference for path integration during nest-centered views, and (ii) whether skylight cues trigger learning-related plasticity in visual pathways to the central complex (CX) and mushroom bodies (MB). Even after block of UV light, polarization information and sun-position the ants were still able to gaze back to the nest entrance indicating that they use a different compass reference for path integration in early outdoor life. However, neuronal plasticity in the CX and MB occurred only under UV with a naturally changing polarization pattern. Whereas passive light exposure triggers pruning of synaptic complexes in visual MB compartments, learning walks under these conditions triggered an increase in synaptic complexes comparable to changes following long-term memory formation. This suggests that celestial cues do not serve as compass reference for path integration during early outdoor life, but a natural skylight-pattern is necessary for learning-related changes in higher visual integration centers indicating that both celestial and landmark cues are learned and memorized during learning walks. Supported by DFG SFB 1047, project B6 to WR.

[P033] Using selective serotonin reuptake inhibitors (SSRI) to investigate the involvement of 5-HT in reinforcing learning in octopuses

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Octopuses display behavioral complexity comparable to that of vertebrates, yet their brain maintains the simpler invertebrate organization. This makes them an exceptional model for exploring the neuronal mechanisms underlying complex behaviors. Previously we reported that serotonin (5-HT) is a powerful facilitatory neuromodulator reinforcing the induction of activity-dependent LTP in the octopus vertical lobe (VL), a brain region involved in memory formation. We now tested whether 5-HT conveys reinforcement signals to the VL during associative learning. Acute exposure to the SSRI, Escitalopram, enhances LTP induction in the VL. Behaviorally, acute exposure to Escitalopram (30µM for 4h) before training on a passive-avoidance task improved the octopuses' short-term learning performance, yet impaired long-term memory tested 24h after training. These results are remarkably similar to those obtained previously following “saturation” of VL LTP by global tetanization of the VL before training (Shomrat et al. 2008). These findings thus support the possibility that SSRI facilitated the 5-HT input to the VL, leading to non-specific LTP saturation during the training. Chronic exposure to SSRI (15µM, 4-hours, 21 days, every other day) improved octopuses' positive reward learning. Our results show the feasibility of using SSRIs to examine the roles of 5-HT in octopus behavior. Furthermore, they specifically support the role of 5-HT as a reinforcing neuromodulator in the octopus learning and memory system. Finally, our findings raise the possibility that residual SSRIs in the maritime environment may affect octopuses' behavior and thus cephalopods' survival.

[P034] Discrete gregarising stimuli elicit serotonin release in the metathoracic ganglion of the Desert Locust

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Serotonin is implicated in the rapid behavioural transformation of the Desert Locust between two distinct phenotypes - a process dependent on population density. At low density, locusts develop into their so-called solitarious phase, in which they are slower and actively repelled by conspecifics. With crowding, locusts transform into their much more active gregarious phase that is attracted to conspecifics. The amount of serotonin measured by HPLC in thoracic ganglia is transiently increased during gregarisation, suggesting a role in the transition to gregarious behaviour, but not its maintenance. However, the specific role of serotonin in regulating phase state remains to be elucidated. We used in vivo fast scan cyclic voltammetry (FSCV) to measure, with high temporal resolution, the release of serotonin following discrete mechanosensory stimuli consisting of single paintbrush strokes applied to gregarising (hind-leg) and non-gregarising (fore-leg, mid-leg, antenna) sites. The recording electrode was positioned in the medial ventral association centre of the metathoracic ganglion. A serotonin-specific voltage waveform was applied to distinguish serotonin from other neuromodulators, primarily dopamine. In both solitarious and gregarious animals, ipsilateral hind leg stimulation caused a larger amplitude release of serotonin than did comparable stimulation of other sites. Stimulation of the antenna also elicited release of serotonin in the metathoracic ganglion but with significantly longer latency. Our study is the first to show that gregarising stimuli in either phase elicit release of serotonin in the central nervous system and demonstrates that serotonergic neurons of gregarious locusts remain responsive to gregarising stimuli.

[P035] Mushroom body-like learning and memory centers across Decapoda

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Pancrustacea is an ecdysozoan clade comprising Hexapoda and Crustacea, whose species occupy a wide range of ecologies and exhibit incredible behavioural diversity. Despite possessing small brains, many of their behaviors are elaborate requiring learning and memory of salient information relevant to their environment. Dicondylic insects and malacostracan crustaceans possess regions of neural circuitry, respectively termed the mushroom body (MB) and the hemiellipsoid body (HB), that constitute second order olfactory neuropils. MBs are iconic centers that have been well-studied in insects, particularly in Diptera and Hymenoptera, and have been shown to play important roles in sensory integration and associative learning and memory. The malacostracan HB is less well studied. However, recent evidence suggests that Stomatopoda, and certain caridid and anomuran malacostracans, possess HBs that share attributes identical to those characterizing insect MBs. It is known that certain brain regions that play similarly important roles have been evolutionarily conserved across different orders of arthropods. However, proposed correspondence of HBs and MBs to an ancestral ground pattern has been contentious. Here we present results of a neuroanatomical survey performed on a wide range of decapod Malacostraca. We demonstrate that species, the behaviors of which suggest developed place memory, possess HBs that share properties of insect MB organization and protein expression. Our results support the hypothesis that HBs and MBs are homologous, retaining an ancestral ground pattern that has undergone exuberant divergent evolution in Malacostraca. While these studies resolve apparent phenotypic homology, in collaboration with another group we plan to investigate their putative genotypic homology using transcriptomics.

[P036] A naturalistic assay of habituation, discrimination, exploration and avoidance at millisecond precision in head-fixed mice

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We have designed a behavioral assay for head-fixed mice that provides a sensitive measure of habituation, discrimination and exploration, as well as avoidance of both conditioned and innately aversive cues. The Virtual Burrow Assay (VBA) simulates a scenario in which a mouse, poised at the threshold of its burrow, evaluates whether to expose itself to potential threats outside or to retreat inside an enclosure. When presented with unanticipated or aversive stimuli, mice exhibit a stereotyped retreat whose onset is marked by a rapid change in the position of a moveable burrow; in some instances this retreat may be preceded by a brief exploratory bout. This abrupt behavioral transition requires no training and unfolds on a millisecond timescale, permitting direct comparison with simultaneously recorded neuronal dynamics. The assay is compatible with standard electrophysiological and optical methods for measuring and perturbing neuronal activity. In preliminary experiments we are assessing its suitability for investigating a diverse set of psychiatric and neurological diseases; pilot data indicate, for example, that the VBA is a sensitive assay of anxiolytics.

[P037] Does the archerfish use motor adaptation to correct for light refraction?

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The archerfish is unique in its ability to hunt by shooting a jet of water at its prey situated above the water level. During a shot, the fish's eyes stay below the water level. In order to hit the target, the fish has to correct for the light refraction at the air-water interface since there is a considerable difference between the actual location of the target and the target's apparent location as seen from below the water level. Despite this distortion, the archerfish routinely hits the target with a high success rate. An open question is whether the ability to compensate for the refraction is a built-in mechanism or is achieved by trial and error through motor adaptation. As a first step towards answering this question, we examined whether the fish can adapt to perturbations in the environment which result in distortion to the shot angle. We trained archerfish to shoot at artificial target situated above the water surface. After several shots, we introduced a perturbing air-flow above the water tank that deflected the trajectory of the water jet from the target. Over the course of several trials, the fish managed to modify its shooting method and successfully hit the target. After the removal of the perturbation, we observed an aftereffect. This result indicates that the fish was generating motor commands that anticipated the perturbing air-flow. Overall, the archerfish is capable of motor adaptation, which suggests that the adaptation process may be the basis for the refraction correction in the archerfish.

Audition

[P038] Hearing in a chicken brain: how is sound localized in space?

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Sound localization is fundamental for survival. Previous research has identified space-specific neurons located in the avian inferior colliculus (IC), which receives input conveying two independent binaural cues: interaural time (ITD) and level (ILD) differences. In the barn owl, ITD and ILD encode information largely about the horizontal and vertical coordinates of sound location, respectively, forming a two-dimensional “space map”. However, such a specialized representation has not been found in any other species. We investigated the plesiomorphic mechanisms underpinning sound localization in the chicken (Gallus gallus) IC under closed- and free-field conditions. Best frequencies of IC neurons were distributed unevenly into low- and high-frequency (> 2 kHz) clusters. Under closed-field conditions, many units showed sensitivity to either ITD or ILD, and nearly half to both. ITD selectivity was disproportionately more common among low-frequency units, while ILD-only selective units were predominantly tuned to high frequencies. We found neurons that displayed bell-shaped ILD tuning, also selective for ITD and typically not frequency selective, similar to neurons in the barn owl’s auditory space map. With free-field stimulation, spatial receptive fields were tuned to frontal, contralateral or both locations. While selectivity in elevation was not obvious, we observed two types of spatial selectivity in azimuth: 1) “hemispheric” fields, mainly in neurons with broad frequency tuning curves and best frequencies above 1 kHz and 2) narrower receptive fields. Our results suggest that generalist birds, unlike barn owls, show a prominent representation of ITD and ILD cues in the IC, providing complementary information for sound localization according to the duplex theory.

[P039] Afferents on hair cells in the foveal region of the barn owl’s basilar papilla: Association between terminals and synaptic ribbons

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Afferent connections with hair cells, whether vestibular or auditory, are of the specialized ribbon synapse type. In the plesiomorphic case, several synaptic ribbons populate one afferent terminal. Mammalian inner hair cells are the exception; these typically show a strict 1:1 ribbon –to- afferent terminal relationship, and many afferent terminals on each hair cell. The barn owl shows a similar but selective increase in numbers of afferent terminals on tall hair cells in the region of its basilar papilla termed an “auditory fovea” (spatial overrepresentation of the behaviourally important high-frequency band 4-10 kHz). Here we explored whether the ratio of ribbons to terminals is unusual in the owl, related to its high-frequency specializations. Basilar papillae were obtained from 8 young adult owls. Serial cross-sections were immunohistochemically labelled to show hair cells (anti-otoferlin or anti-MyVIIa), synaptic ribbons (anti-CtBP2), all neural structures (anti-Na+-K+-ATPase) and efferent terminals (anti-synapsin). Individual hair cells were systematically sampled from different locations along and across the papilla with confocal microscopy. The number of ribbons per hair cell declined from neural to abneural, consistent with the known decline of afferent innervation density from tall to short hair cells. In tall hair cells, 1-4 ribbons typically faced a given afferent terminal. There was no clear trend with tonotopic position or the total number of terminals on a given hair cell. Thus the number of synaptic ribbons per terminal is not altered in the specialized foveal regions of the basilar papilla. Ongoing work aims to quantify the dimensions of synaptic elements.

[P040] Vocalizations as auditory objects: Cues for simultaneous auditory grouping in green treefrogs

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Animal acoustic communication often occurs in noisy social aggregations (e.g. songbird dawn choruses, a penguin crèche, or frog and insect breeding choruses). A fundamental challenge encountered in such environments is the requirement that receivers assign sounds to their correct sources, a perceptual process referred to as "auditory grouping." Because animal acoustic signals are rarely considered from the perceptive of perceptual auditory object formation, we have limited knowledge of the cues and mechanisms that allow receivers to perform auditory grouping. We tested the hypothesis that females of the North American green treefrog (Hyla cinerea) exploit onset/offset synchrony and common spatial origin as perceptual cues for grouping separate formant-like spectral components in their advertisement calls. This is a particularly relevant question for frogs, because the two spectral components are primarily transduced by different sensory papillae in the inner ear, such that grouping requires neural computations by the central auditory system. Using two-alternative choice tests, we found that calls with both spectral components are more attractive than calls with only a single component, even when controlling for overall amplitude. Introducing onset/offset asynchrony greater than 25 ms between the two spectral components rendered asynchronous calls less attractive than alternatives with synchronous components. Two synchronous components separated by more than 45° in azimuth were less attractive than spatially coherent alternatives. Together, these data are consistent with the hypothesis that both temporal synchrony and spatial coherence promote auditory grouping in green treefrogs. The contribution to synchrony-based grouping of combination-sensitive neurons in the midbrain is under investigation.

[P041] Auditory temporary threshold shifts under acoustic stress in zebrafish

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Exposure to persistent moderate sound levels is known to cause auditory fatigue leading to temporary (or even permanent) threshold shifts (TTS), which may impact the overall physiology of different vertebrates, including humans. The mechanisms underlying TTS are not well understood and limited information is available on fish that share homologous inner ear structures to mammals. Zebrafish (Danio rerio) has become an important model system to study the development and function of the vertebrate inner ear, the genetic causes of hearing loss, and the molecular players of hair cell regeneration. However, the impact of noise exposure on zebrafish auditory sensitivity and associated TTS has never been investigated, yet it occurs in other fish, especially in species with morphological hearing specializations. Here, we examined the effects of continuous noise conditions on zebrafish auditory sensitivity thresholds. Specimens were exposed for 24h to white-noise (c. 140 dB re 1 µPa) versus quiet conditions (c. 105 dB re 1 µPa), and their auditory sensitivity subsequently measured through the auditory evoked potential (AEP) recording technique. Results revealed significant TTS of 12-18 dB within the best hearing frequency range (400-2000 Hz) and increased response latency for the noise-treated group. Furthermore, the AEP recordings depicted changes of specific waveform components providing further evidence of dysfunction of the auditory neural system under acoustical stress. Current research focuses on auditory sensitivity recovery, identification of potential changes in the inner ear sensory morphology and underlying molecular mechanisms of TTS in zebrafish.

[P042] Sound localization performance by a gleaning bat, Antrozous pallidus

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Gleaning bats face a problem analogous to the barn owl's – hunting based on soft walking sounds in darkness. Due to this, it was speculated that the pallid bat, an obligate gleaner, must have a sophisticated sound localization system. Many studies have since focused on characterizing the neuroethology of sound localization in the pallid bat. Here, we trained pallid bats on an azimuth localization task wherein the head position and sound bandwidths were controlled. Bats localized a broadband noise (5-30 kHz) that mimicked prey-generated noise from 1 out of 11 speakers, evenly-spaced across the bat's frontal hemisphere. Based on this task, we report that the pallid bat shows remarkably accurate localization ability, averaging errors of 3-6 degrees, which is comparable to the barn owl. Use of filtering to reduce the bandwidth of the noise to include just 20-30 kHz, or 5-15 kHz shows that higher frequencies (20-30 kHz) are necessary for the accurate localization behavior. This is consistent with electrophysiological and ear directionality studies that show that interaural intensity differences play a critical role in localization in the pallid bat. Taken together, these studies indicate that the pallid bat is a suitable system to study neural mechanisms of sound localization. Ongoing research aims to determine the impact on behavior of selective and reversible lesions within the cortex, utilizing pharmacogenetic techniques. Additionally, performance on an elevation localization task is currently being assessed.

[P043] Effects of acoustic stimuli on the behaviour of wild and captive shark

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The effect of sounds on the behaviour of sharks has not been investigated since the 1970s. However, at a time when mitigation strategies are in high demand to counter fisheries pressure on sharks caught as bycatch, and negative interactions between sharks and humans, sound offers an advantage over other sensory stimuli, as it can spread in all directions quickly and further than any other sensory cue. We investigated the behavioural responses of wild and captive sharks to the playback of two different sounds: killer whale calls and a custom-made artificial sound. We also presented a combination of the sound with bright flashing (strobe) lights to explore the effect(s) of multisensory cues. Great white sharks (Carcharodon carcharias), as well as seven species of benthopelagic reef sharks were targeted in the wild, while Port Jackson sharks (Heterodontus portusjacksoni) and epaulette sharks (Hemiscyllium ocellatum) were tested under controlled conditions in the laboratory. Our results show interspecific differences in the effect of underwater sounds on shark behaviour. We also found an enhanced adverse effect when strobe lights were used in combination with sound. We discuss our results within a neuroecological framework, in the context of anthropogenic noise and shark mitigation technologies.

[P044] Who needs impedance matching? Mechanisms of air-borne hearing in recent and early non-tympanate tetrapods

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Tetrapod tympanic hearing probably emerged in the Triassic with independent origins in each of the major tetrapod groups, more than 120 my after the tetrapods became terrestrial. This shows that during this long period auditory sensitivity must have been based on non-tympanic, bone conduction mechanisms. However, bone conduction is a loose common term for several different modes of vibration that can stimulate the inner ear. To understand hearing in a non-tympanic ear, I will focus on the simplest: that sound translates the head region and that this vibration is transduced by the inner ear. According to theory, the efficiency of translation of an object by sound is determined by ka (the product of wave number and radius). I will present data from animals without middle ear (snakes, salamanders, earless frogs, lungfish). Comparison of their sensitivity to sound and to vibrations of the skull shows that most of the sensitivity can be explained by translation. Interestingly, simple translation is also the mode of human low-frequency bone conduction sensitivity (for frequencies resulting in a ka>1). The data will also be compared to simple models of translation by sound. Translation by underwater sound is the mode of stimulation of most fishes. It is therefore a straightforward assumption that this was the mechanism of hearing also in the early tetrapods. How translation of head or body is stimulating the inner ear is naturally most evident in an inertial system like the fish and early tetrapod otolith/otoconial ear, but fluid inertia in the inner ear may also generate hydrodynamic waves that can stimulate hair cells.

[P045] Effects of acoustic overstimulation on otoacoustic emissions generated by the amphibian ear

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The two auditory organs in the amphibian ear, the amphibian papilla (AP) and the basilar papilla (BP), differ not only in sensitivity and frequency tuning but also in their physiological vulnerability. Distortion-product otoacoustic emissions (DPOAEs) generated from the AP are more susceptible to physiological insult than are those from the BP. Similar differences are expected in DPOAE responses following acoustic overstimulation. This study aims to analyze the susceptibility of the amphibian inner ear to high-level stimulation by evaluating DPOAE generation. DPOAE audiograms and DPOAE input/output functions were determined in Rana pipiens. The frequency 2f1-f2 of highest emission level was identified in the spectral range corresponding to each papilla to design the acoustic overstimulation paradigm. High-level stimuli (100 dB SPL) were delivered for two hours using either pure tones or 1/3-octave-band noise. DPOAE measurements were taken before high-level stimulus exposure and 15 min, 1, 2 and 24 h post-exposure. DPOAE-audiograms exhibited a bimodal shape with low- and high-frequency peaks representing DPOAEs generated within the AP and the BP, respectively. After 2 hours of overstimulation, DPOAEs dropped to or near noise floor levels. Input–output curves also revealed drastic changes in sensitivity. DPOAE shifts typically returned to pre-exposure levels after 24 h. The relatively short time required for complete recovery suggests there was minimal damage to the sensory epithelium, but metabolic alterations following acoustic trauma could be responsible for the observed changes in these nonlinear responses.

[P046] Spectral tuning of synaptic inhibition in duration-tuned neurons from the bat auditory midbrain

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Duration-tuned neurons (DTNs) are formed by the interaction of excitatory and inhibitory synaptic inputs. We used single-unit recording and paired-tone stimulation to measure the frequency tuning of inhibition in DTNs from the inferior colliculus of the big brown bat (Eptesicus fuscus). Cells were stimulated with a roving, short duration, excitatory probe tone set to the best duration (BD) and best excitatory frequency (BEF), and a stationary, longer duration, non-excitatory (NE) suppressor tone whose frequency was varied. In the monotic condition, both tones were broadcast to the contralateral ear and when they were close in time the NE tone suppressed BD tone-evoked spiking in every cell. Also, contralateral inhibition preceded the cell's excitatory first spike latency (FSL).

In the dichotic condition, the BD tone was broadcast to the contralateral ear and the NE tone to the ipsilateral ear, and when they were close in time spike suppression did not occur in every cell. Moreover, ipsilateral inhibition lagged the FSL. Monotically, inhibition was strongest when the NE tone frequency fell within the excitatory bandwidth (eBW), and the duration of spike suppression decreased for NE tone frequencies outside the eBW. In both conditions, the onset of inhibition did not vary with NE tone frequency, and best inhibitory frequencies matched BEFs. Monotically, the inhibitory BW was wider than the eBW, whereas dichotically the opposite was true. The data indicate that the temporal selectivity of DTNs is created with contralateral (monaural) inhibition, with ipsilateral (binaural) inhibition perhaps shaping a neuron's spatial tuning.

[P047] The Difference a Day Makes: Oviposition Influences Endocrine State and Peripheral Auditory Processing

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For many animals there are rapid physiological and behavioral transitions that occur when females oviposit. In many species of treefrogs, gravid females actively search out and approach males based on the quality of their calls. Therefore, female preferences can be easily assessed using phonotaxis experiments. However, immediately after oviposition female responsiveness to male calls decreases dramatically and females will no longer respond to male vocalizations. In this study we asked whether there are concomitant changes in circulating hormone levels and the sensitivity of the auditory periphery to common elements of male vocalizations. In this talk I will discuss our surprising result that peripheral auditory sensitivity, in some cases, appears to increase following oviposition; while, unsurprisingly, circulating steroid levels drop dramatically following oviposition. However, the relationship between peripheral processing and circulating hormone levels is not straightforward. Therefore, it is not yet clear whether changes in auditory processing are the result of hormonal changes, metabolic changes, or changes in motivational state that may influence background physiological noise and response to paralytics. Future work will focus on teasing apart these effects to understand the processes that mediate the dramatic behavioral changes across the 24 hours in which oviposition occurs.

[P048] Articulatory movement associate sound and meaning in human

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Sound symbolism is the idea that a sound makes a sort of impression (e.g., phoneme "p" is associated with small impression) and it could serve as a psychological basis for the word–meaning association. Previous studies suggested that articulatory movement contribute to sound symbolism. The purpose of this study is to clarify the involvement of articulatory related brain region in the sound symbolism. In the experiment, subjects were required to compare the visual size between standard and target stimulus while listening to syllables assumed to have either larger or smaller impression. They performed the task under functional magnetic resonance imaging (fMRI) scanning. Congruent trials were defined as those where circle sizes were correlated with the impression of accompanying sounds, and incongruent trials were with opposite stimulus contingency. Additionally, the contribution of articulatory movement to the sound symbolism was investigated by a localizer experiment, where subjects were required to pronounce phonemes aloud and to pronounce phonemes without aloud under fMRI scanning. As a result, reaction times in incongruent condition were longer than congruent condition suggesting that sound symbolism was observed between visual size and syllables under fMRI. We will discuss relationships between the activation area for sound symbolism and articulatory related brain regions: the activation area for articulatory movement and phoneme perception.

[P049] The auditory world of lizards

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The Australian fauna is rich in lizard species, and a number of these have been the subject of studies of their auditory physiology. These include especially the Bobtail skink or "sleepy lizard", but also small geckos, including the uniquely australasian pygopods. Studies of these species can be compared to non-Australian species to obtain a more global insight into their auditory senses.
In general, lizards hear remarkably well, with a sensitivity close to that of humans, at least at low frequencies. There are, however, clear differences between lizard families, and, in all cases, the upper frequency limit depends on the body temperature, rising with a temperature Q10 between 0.025 and 0.06 per degree. Whereas lizards of some families (agamids, skinks, varanids) have upper hearing limits near 4 to 5 kHz, others, such as small gekkonids, hear up to 6 to 7 kHz. A clear exception are pygopod geckos of the genus Delma, whose upper limit is nearer 14kHz.
Despite these impressive hearing abilities, behavioural relevance for communication has only been documented for geckos, which of course correlates with their nocturnal habits. Nonetheless, many other lizards change their activity patterns from diurnal to nocturnal during the summer heat, yet have not been reported to use

vocalizations to communicate. An especially rewarding group for future study would be the pygopods, that are generally nocturnal and very readily vocalize when handled, using broad-band signals that cover their high-frequency hearing range and in some cases avoid frequencies readily heard by birds.

[P050] Impact of head morphology and natural postures on sound localization cues in crocodylians

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As top predators, crocodylians are well equipped to probe their environment: excellent vision in air, highly developed smell, extraordinary use of mechanoreceptors, and an acute sense of hearing. These sensory abilities are extremely useful to localize their preys and their congeners: individuals interact visually, chemically and acoustically. The acoustic environment of crocodylians is complex because both hunt and social behaviours occur at the interface between air and water. One way to investigate sound localization abilities of crocodylians consists in characterizing how their ears receive external auditory cues, i.e. the “Head-Related Transfer Functions” (HRTF). We have measured HRTFs using microphones placed inside the ears of young living crocodylians, skulls and stuffed animals because of the difficulties of experimentation on adult individuals. The main objective of our work was to characterize the HRTFs in two different natural body positions: resting on the ground and floating at the waterline interface. As other Archosaurs, the ears of the crocodylians are linked by an interaural air canal, which could play a part in the localization cues. The results highlight the presence of external sound localization cues in both natural positions. These cues could be used by crocodylians to accurately estimate the position of sound sources. Body size influences these cues, with bigger crocodylians showing a low frequency shift. Measurements on skulls allowed a modelling of the interaural canal influence on HRTF cues. Combined with virtual reality technology and signal processing, this dataset allowed some behavioural experiments to confirm the relevance of HRTFs for sound localization.

[P051] Response mode choice in a multimodally duetting paleotropical pseudophylline bushcricket

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Females of the pseudophylline bush cricket species Onomarchus uninotatus respond to a conspecific acoustic call with bouts of tremulation for a median period of 2 minutes, followed by phonotaxis in some cases. This tremulation sends out a vibratory signal that propagates along the branch on which the female sits, and the male has been shown to be able to localise the signal and perform vibrotaxis towards the female if he is on a connected substrate. Males are unable to localise the signal if it emanates from a branch unconnected to their perch, and therefore, female tremulation might not be a productive response leading to mutual localization when the nearest male is on an adjacent, disconnected tree. We hypothesised that features of the male call that indicate caller distance might affect the female's behavioural choice between tremulation and phonotaxis. Since low SPL calls might indicate that the male is calling from further away, increasing the probability that the caller is on another tree, we hypothesized that the choice between phonotaxis and tremulation would vary with call sound pressure level (SPL). We found that at all SPLs, the female tremulates before attempting phonotaxis, and that tremulation rate increases with song SPL. Following tremulation, females perform phonotaxis with a probability that is directly proportional to call SPL. However, the strength of the vibratory signal produced by tremulation and its attenuation with distance does seem to affect the mode of localization between vibrotaxis and phonotaxis.

[P052] A comparative study of stimulus-specific adaptation in an auditory neuron in Neotropical katydids (Orthoptera: Tettigoniidae)

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In many katydid species, males produce repetitive acoustic signals to attract females. Katydid ears are broadly tuned to detect both male acoustic signals and the ultrasonic echolocation calls of predatory bats. Previous studies have shown that an acoustic interneuron, TN-1, exhibits stimulus-specific adaptation to different frequencies of sound. This property allows TN-1 to encode the rare echolocation calls of bats while being unresponsive to repetitive, lower-frequency background noise. Stimulus-specific adaptation has so far been studied in katydid species that produce highly repetitive calls, often in a chorus of other males where this property would be especially adaptive. In Neotropical forests, however, there is enormous variation across katydid species in the carrier frequency of the male call (including many ultrasonic species), the repetition rate of male calling, and the location of calling (from the understory, where background noise from crickets is significant, to the relatively quiet canopy). We recorded TN-1 activity in 15 Neotropical katydid species from three subfamilies to test whether stimulus-specific adaptation is 1) a conserved feature in katydids or 2) varies with species-specific behavioral and ecological traits. For each species, we also measured acoustic features of the male call, calling rate over 24 hours and the height of calling within the forest canopy (based on recordings from acoustic monitors placed at three levels in the forest canopy) and tested for correlations between these traits and the strength of stimulus-specific adaptation across species.

[P053] Titrating the effect of low-frequency sound on the mammalian cochlea

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Low-frequency sound caused by anthropogenic or natural sources travel long distances and are ubiquitous in our environment. Mammalian ears comprise two populations of sensory cells, inner and outer hair cells. Outer hair cells respond strongly to low-frequency sound and are known to amplify sound and, as a by-product, to generate otoacoustic emissions. In humans and guinea pigs, temporary sensitivity changes of the inner ear were shown in response to low-frequency sound at high intensities. Indices for those typically cyclic sensitivity changes were, amongst other measures, alterations of hearing threshold and changes in level of otoacoustic emissions. In the present study, we aim to investigate the impact of intense low-frequency sound on another mammalian species with rather low-frequency hearing and particularly large otoacoustic emissions, the Mongolian gerbil (Meriones unguiculatus). Distortion product otoacoustic emissions are recorded in anesthetized animals before and after low-frequency stimulation (30-500 Hz, 110-120 dB SPL) with different exposure times lasting from 30 seconds up to 1 hour. Amplitudes of otoacoustic emissions are analysed after low-frequency sound exposure focusing on quadratic distortion products which were previously shown to be prone to low-frequency induced changes. With regards to the different exposure times, we are particularly interested in whether and when temporary sensitivity changes will transition into a permanent damage of the inner ear. Acknowledgements
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[P054] Mate or escape: mosquitoes analyze the frequency of sound to make a choice

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Our knowledge of the mosquito behavioral responses to sound is primarily based on the experiments modeling male-female interactions. In one design, male mosquitoes are attracted to the sounds which resemble the wing-beat frequency (WBF) of a conspecific female. In the other, the experiments involve rapid frequency modulation, an acoustic 'dance' which occurs in a male-female pair immediately prior to mating. Probably due to thus focused experimental designs, little is known about behavioral responses other than reactions to the sound of a mate. At the same time, our recent findings on the frequency selectivity among the highly numerous auditory neurons in the mosquito ears, the Johnston's organs, suggest that mosquitoes can potentially demonstrate different kinds of responses to different frequencies of sound. Here we demonstrate that tonal acoustic signals in the range of 140–200 Hz induce a repellent effect in male mosquitoes Aedes diantaeus (Diptera, Culicidae). Mosquitoes which were previously attracted by the sound imitating the WBF of a female (280–320 Hz) left the stimulation area within one second from the onset of the test signal (amplitude 57–69 dB SPVL). Such differentiated response – positive or negative phonotaxis – strongly suggests that mosquitoes use frequency analysis of sound to make a behavioral choice.

Vocalisation

[P055] Midbrain coding of vocal behavior in teleost fish

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Teleost fish vocalizations are rapidly produced repetitions of brief sound pulses. Midshipman toadfish have 3 call types: long duration (secs-mins) “hum” advertisement calls with highly stable pulse repetition rates (PRR); brief (≤ 200 msec) agonistic “grunts” that occur singly or as stable, repetitive series (“train”); agonistic “growls” that occur singly or repetitively, but show variable duration, PRR, and amplitude. A descending vocal control system comparable to that in birds and mammals drives a hindbrain vocal central pattern generator (CPG). Vocal CPG output, the vocal nerve motor volley, is easily recorded in-situ as a fictive call that directly predicts call duration, rhythmicity (i.e. PRR) and amplitude on a msec timescale. Electrical microstimulation (E-ms) implicates midbrain sites in call initiation, including a region considered a homolog of the tetrapod periaqueductal gray (PAG). Prior studies using E-ms typically evoked fictive grunts in lateral tegmentum regions adjacent to the ventricle (collectively referred to as the PAG), other tegmental sites, or the nearby medial longitudinal fasciculus (MLF). These studies also showed that the PAG is a key node linking the hypothalamus and vocal CPG. We can now evoke more complex fictive hums and grunt or growl trains for periods lasting 90-180 min from the PAG and/or hypothalamus via microinjections of glutamate or gabazine, a specific blocker of the inhibitory GABA(A) receptor. Such effects are not seen after injections into the vocal CPG or MLF. The results provide essential support that a

local midbrain population, the PAG, forms an essential link between the hypothalamus and vocal CPG in teleost fish. Research support from US NSF (IOS1656664).

[P056] Premotor cortical control of learned and innate vocalizations in interacting zebra finches

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Many animals use innate vocalizations to interact with conspecifics, while only a small subset of species possess the ability to produce learned vocalizations, such as speech or birdsong. It is unknown whether and how premotor cortical areas are differentially involved in the production of innate and learned vocal outputs. We address this issue in male zebra finches, which produce a variety of innate vocalizations alongside their learned courtship song. The forebrain premotor nucleus HVC underlies the production of learned song by providing experience-dependent, developmentally refined neural control via temporally precise firing patterns. Additionally, recent studies of these birds' short non-learned calls have implicated the forebrain target of HVC in call production and coordination. Here, we used an intracellular microdrive to record from HVC projection neurons and interneurons in vocally interacting males. We found evidence that HVC neurons can serve multiple functions, in the production of various innate calls in addition to learned song. This HVC activity exhibited temporal bias toward call initiation. Specifically, cells either increase their firing or were actively suppressed prior to call onsets, suggesting a close interplay between premotor neurons and inhibitory interneurons. The resultant triggering of calls as discrete gestural units contrasts with premotor activity during singing, which is less strictly bound to syllable onsets. Our findings begin to reveal the cortical dynamics governing the timing of learned versus innate vocalizations, expanding the role of the premotor nucleus HVC beyond production of learned song to include the temporal coordination of non-learned vocal signals.

[P057] Interspecies communication of distance in the rattlesnake acoustic threat display

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Some animals make use of acoustic threat displays to deter other animals from preying or stepping on them. Rattlesnakes produce broadband acoustic sounds (rattling) as an aposematic signal to honestly warn approaching animals. This sound is generated by the superfast tailshaker muscles at rattle frequencies of up to 120 Hz. Interestingly rattlesnakes can control their rattle frequency over a noticeable range. Here we tested the hypothesis that the rattle frequency is used to convey distance information to the receiver. We presented visual looming stimuli at different velocities and magnitudes to Western-Diamondback Rattlesnakes (Crotalus atrox) and recorded their rattling responses. Rattlesnakes readily responded to visual stimuli from which the rattle frequency and amplitude were extracted for data analysis. We found that the rattle frequency possesses two distinct ranges, a low range (1 to 30 Hz) and a high range (70 to 120 Hz). Snakes started their acoustic display to the visual looming stimuli in the low frequency range and increased rattle frequency dependent on stimulus velocity until they abruptly switched into the high rattle-frequency range, in which no correlation to stimulus size or distance could be detected. Our experiments suggest that rattlesnakes use the low rattle-frequency range of their acoustic threat display to communicate their distance from their addressee, while the abrupt switch to the high rattle-frequency range is used to falsely convey a too-close distance.

[P059] Are adult bats capable of vocal imitation?

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The neuronal and genetic underpinnings of human language acquisition are still poorly understood, despite major advances in many fields pertaining to neurobiology. In order to gain insights into the human capacity for language learning, animal model systems are employed. Historically, vocal learning, i.e. the capacity of some animal species to imitate sounds outside their innate vocal repertoire, has mainly been studied in songbirds. However, to further the understanding of this language-relevant trait, comparative studies, especially including mammalian species, are of great importance. A mammalian model system for vocal learning that recently has shown promising results is bats. We established a strictly controlled behavioural paradigm that allowed us to investigate vocal learning in adult pale spear-nosed bats. Using an automated setup and a multi-stage training plan we were able to track spectral and temporal changes in vocalisations of individual isolated bats in response to pitch-shifted playbacks and a selective reward regime. Six bats were successfully trained to produce communication calls for food reward in isolation. Once the animals had learned this task, a downward frequency-shifted template call was presented when the bats broke a light barrier. Bats were further encouraged to lower their call frequency by rewarding them dependent on the magnitude of their imitated frequency shift. Over the course of 60 - 90 training sessions, five out of six bats indeed shifted their fundamental frequency significantly downward. Ongoing experiments are investigating adult bats' ability to imitate fundamental-frequency contours, assessing in more detail their suitability as animal models for the study of vocal learning.

[P061] Encoding of learned vocalizations in the developing mammalian brain

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Infants and children are expert vocal learners; however, the neural mechanisms supporting vocal learning in mammals have never been studied before due to the remarkable sparsity of vocal learning capabilities across mammalian species. To address this knowledge gap we study vocal learning in one of the only known non-human vocal learning mammals: bats, specifically, the Egyptian fruit bat (Rousettus aegyptiacus). We chose bats not only because of their mammalian brain organization, but also for their impressive social vocal repertoire and learning capabilities. Previous studies in bats have focused primarily on perception of acoustic signals and not on production of learned vocalizations. Furthermore, no study to date has examined the neural computations which might support vocal production in a developing mammal. Here we describe the first efforts toward studying the mechanisms that support vocal production and learning in a developing mammalian brain during free, naturalistic behavior. We used lightweight wireless electrophysiology to study the activity in the frontal motor cortices of juvenile bats engaged in natural social interactions. We found that a substantial fraction of neurons exhibited responses during production of learned vocalizations, but not during auditory playback. These responses were independent of both the temporal ordering and acoustic structure of the produced vocalizations, but were tightly locked to the onset of learned vocalizations. These results suggest an encoding of a “go-signal” for the production of learned vocalizations. Combined, we present here the first evidence of cellular-resolution neural activity related to the production of learned vocalizations in the developing mammalian brain.

[P062] Establishing the behavioral, anatomical, and neurophysiological foundations for studying vocal learning in a mammalian model system

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Vocal learning is a complex motor production skill that leverages sensorimotor integration to modify a vocalization. Discovering the neural mechanisms underlying this ability in the mammalian brain is crucial to our understanding of human proficiency in speech learning. However, the absence of a tractable mammalian model system to probe this behavior has been a major obstacle to this end. Egyptian fruit bats are believed to acquire their social communication calls through the process of vocal learning. To study these faculties in the bat, we must address two fundamental needs: a reproducible vocal behavior, and insight into the neural circuits involved in vocal learning. We tackle these issues through a combination of behavioral, genetic, anatomical, and imaging techniques. We developed a custom fully-automated operant behavioral setup using real-time audio processing and food reward to motivate vocal production in adult bats, thus allowing us to obtain a large sample of highly reproducible voluntary vocal production signals within a single behavioral session. Subsequently, we began working towards implementing the system to stimulate vocal plasticity in adult bats with modification of vocalizations through operant conditioning. In parallel, we used comparative gene expression profiling and tract-tracing to anatomically identify a direct corticobulbar pathway believed to be unique to vocal learners. Finally, we are pursuing freely-behaving calcium imaging methods to track responses of large populations of neurons in cortical networks during vocal production and learning. Collectively, we will present our efforts towards establishing the appropriate behavioral, anatomical, and neurophysiological foundations for studying vocal learning in a mammal.

[P063] The neuroethology of bat songs

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Echolocating bats are unique in their reliance on voice to survive. Their highly cognitive biosonar behaviors and the recent revelations that many bat species sing like birds offer a challenging exception to what we think we know about how the mammalian brain is wired to communicate. Their brains are adapted to rapidly and precisely manipulate the sound of their voice to meet the ever-changing environmental and contextual demands of sonar navigation. Complex vocal behaviors such as sonar, singing and human speech require highly developed forebrain networks that seamlessly translate motivation and cognition into the pliable coordination of respiratory, laryngeal and supralaryngeal vocal motor commands. To achieve this, bats, primates and cetaceans may have converged upon similar neuroarchitectural design solutions, but for completely different ecological reasons. Here I first review what's unique about bat vocalizations, what's currently known about their neural substrate, present new evidence of which forebrain networks are active during song production, and discuss how this informs hypotheses about how vocal motor pathways underlying complex vocalizations may have evolved.

Echolocation

[P065] Can sound paint a picture? Biosonar perception of spatial and temporal frequency

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Biosonar as bats' main remote sense to image space comes with drawbacks. Firstly, bats must construct a spatial representation from binaural echo features as the bat cochlea represents echoes along an audio-frequency axis, not a space-axis like the retina. Secondly, the sparse temporal sampling of the environment impairs the assessment of changes, as for example in the distance of an object over time. For fast periodic changes an echolocating bat may run into aliasing problems, while slow changes can only be perceived by tracking echo features along sequences of call-echo pairs. In two formal psychophysical experiments with the bat Phyllostomus discolor, we have systematically evaluated 1) biosonar sensitivity to spatial frequency (change as a function of space) and 2) sensitivity to temporal frequency (change as a function of time). Bats were trained to discriminate between two targets. In experiment 1), the reference target had a spatial frequency of 0 cycles/m, corresponding to a flat surface, while the test target was covered with waves of varying spatial frequencies and amplitudes. Our results indicate that biosonar in bats is more sensitive to higher than to lower spatial frequencies, implying that echolocating bats can segregate prey from background according to spatial frequency. In experiment 2), the reference target had a temporal frequency of 0 Hz, corresponding to an stationary target, while the test target's distance was modulated at varying temporal frequencies. Bats reveal conspicuous modulation sensitivity: good sensitivity at low and high modulation frequencies and worse sensitivity for intermediate modulation frequencies around 20 and 50 Hz.

[P066] Acoustic underpinnings of the effectiveness of sonar jamming behavior in Bertholdia trigona

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Jamming signals from tiger moths should be produced in a very narrow time window, just before the arrival of an echo to degrade bat sonar. Bertholdia trigona increases the probability of this time coincidence by producing clicks at high duty cycles (DC). Their clicks are also frequency modulated. The dominant frequency sweeps from high to low frequencies within the active half-modulation cycle (HMC) and back again during the passive HMC. Little is known about the effect of DC and the direction of frequency sweeps, in the effectiveness of the jamming signal. This study combines behavioral and neurophysiological experiments that evaluate the effect of the frequency-time properties of moth signals over bat's foraging performance and neural processing disruption. We recorded the echolocation behavior of big brown bats (Eptesicus fuscus) attacking tethered moths in a flight room, under the effect of B.trigona signals with different DC (5% – 45%). High DC signals were more effective at deterring bats performance. Neuron activity in the inferior colliculus of E. fuscus was recorded in response to stimuli combining echolocation calls and B. trigona signals. The temporal pattern of the neural response to search and approach signals was disrupted in the presence of modulation cycles produced at high DC. The direction of the frequency sweep also influenced the neural firing pattern. These results suggest that moths may be using a "sweep jamming" strategy that requires a minimum DC to makes their defense more effective.

[P067] Echolocating bats adaptively change acoustic characteristics of their vocalization depending on time – frequency structures of jamming sounds

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When flying in a group, echolocating bats have to separate their own echoes from pulses and echoes belonging to other individuals to extract only the information necessary for their own navigation. The mechanism of jamming avoidance in such acoustic interference environment has been incompletely understood. Previous studies demonstrated that frequency modulated (FM) bats change the terminal frequencies (TFs) of downward FM pulses according to the degree of acoustic interference. In this study, we examined the changes in the acoustic characteristics of pulses emitted by bats by presenting five types of jamming stimuli with different time frequency structures to the bat flying alone. The stimuli were constant frequency sound (CF), downward exponential FM sound (Exp) like echolocating sounds of bats, downward linear FM sound (Lin), time-reversed version of Exp (rev-Exp) and time-reversed version of Lin (rev-Lin). Echolocation pulses were recorded using a telemetry microphone mounted on the backs of bats. We investigated how bats recognize the difference in time frequency structure in context of jamming avoidance. As a result, the bats shifted their TFs by 1–3.5 kHz in response to the Exp, rev-Exp, and CF jamming signals within 150–200 ms after stimulus onset. On the other hand, they didn't shift their TFs in the presence of the Lin and rev-Lin jamming sounds. These results suggested that echolocating

bats rapidly change their TFs depending on the frequency structures of the jamming sounds and the changes in TFs were more remarkable when the jamming sounds interfered frequency ranges around their TFs.

[P068] Do echolocating bats give a foraging patch to the next bat for optimal foraging?

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The purpose of this study is to investigate the aerial foraging behavior of echolocating bats in the field. We measured ultrasounds and flight paths of Myotis macrodactylus using 16-ch microphone-array system during foraging over the pond sizing approximately 20 meters square in June and September, 2016. By surrounding the entire pond with the microphone-array, we could acoustically determine the foraging duration of individual bat as well as the number, the timing and 3D position of prey capture by the bat. As a result, a total of 39 and 63 bats appeared during approximately one hour in June and September respectively, and gained profits corresponding to the foraging duration at the pond. The bats usually foraged alone in different time zone and stayed at the pond longer in June (76.4 ± 84.2 s) than in September (41.7 ± 30.9 s) (mean ± SD). We found that most of the bats (80%) left the pond immediately after another bat came to the pond. This indicates that the bats make a decision to give a foraging patch to the next bats. These results imply that Myotis macrodactylus achieve the cooperative foraging by giving the multiple foraging patch each other. [This research was supported by a Grant-in-Aid for Young Scientists (B), Scientific Research (A) and Scientific Research on Innovative Areas of JSPS, and the JST PRESTO program.]

[P069] Bats decrease the similarity of their calls to solve the problem of signal jamming by conspecifics

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Echolocating bats perceive surrounding environments by hearing echoes of self-generated ultrasound pulses. When they swarming, pulses and echoes of other conspecifics flying together result in chaotic acoustic situation. To understand how echolocating bats process their echoes of interest in the presence of conspecific sounds with similar acoustic features to own sounds, we observed echolocation behavior of bats flying in a small group (consisting of four bats), by recording echolocation pulses emitted by each individual of the group. As a result, the bats significantly increased differences in their terminal frequencies (TFs) of the emitted pulses by 0.5 kHz during Group flight in comparison with Single flights 1 and 2 (Tukey's HSD test, $P < 0.05$). Also, similarities of pulses between individuals of the groups significantly decreased from 0.32 ± 0.12 and 0.29 ± 0.12 in Single flights 1 and 2, respectively, to 0.21 ± 0.07 in Group flight (Tukey's HSD test, $P < 0.05$). Moreover, to understand what features most affect the similarity between pulses, we calculated the similarity of signals mimicking pulses, and found that the similarity between those artificial signals was decreased most with slight manipulation in TF in comparison with other characteristics, like start frequency or duration. These results demonstrate that signal jamming problem is solved by this simple strategy, which may be universally used by animals that use active sensing, such as echolocating bats and electric fish, transcending species and sensory modalities.

[P070] Echolocating bats modulate sonar emission rates based on interaction with other individuals

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The purpose of this study is to understand how bats modulate their echolocation behavior to reduce acoustic interference from sounds of conspecifics flying together. We made Japanese horseshoe bats (Rhinolophus ferrumequinum nippon) fly singly and in pairs. Echolocation pulses emitted by each individual during paired flight were captured with individually mounted on-board microphones. Video recordings were made in order to reconstruct the three-dimensional coordinates of each bat. Since the bats during paired flight typically flew in a circle, we divided their flights into two patterns, same direction and reverse direction flight, based on the rotation direction of one bat relative to the other bat. As result, interpulse interval (IPI) was increased with the decreasing inter-bat distances in same direction flight. In this flight, the mean IPI was increased from 60.5±8.9 ms to 74.1±14.3 ms when the inter-bat distance was decreased from 5.0-5.5 m to 2.0-2.5 m. On the other hand, in the reverse direction flight, mean IPI was decreased with the inter-bat distance decreased. Mean IPI was decreased from 85.8±18.7 ms to 42.4±18.1 ms when the inter-bat distance was decreased from 3.5-4.0 m to 0-0.5 m. These results suggested that a bat decreases pulse emissions in order to listen to sounds of the other bat in same direction flight, and increases sensory acquisition rates to frequently know exact position of the other for collision avoidance in reverse direction flight.

[P071] Mechanisms of acoustic interference in echolocating bats

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Many bat species rely on the use of frequency-modulated (FM) biosonar emissions to obtain crucial information about the environment. This mode of sensing, however, leaves bats susceptible to acoustic interference, or sonar jamming, and reduces the efficacy of echolocation. Though numerous studies have documented the effects, of various acoustic stimuli, it remains unclear what makes a jamming signal effective. We herein investigate whether sweep direction plays an important role in sonar jamming by presenting variations of naturally occurring bat calls to foraging individuals and compare the capture rates of tethered moths along with several call parameters of big brown bats (Eptesicus fuscus). Further, we discuss the implications observed echolocation changes for jamming avoidance responses and examine the potential neurophysiological mechanisms associated with sonar jamming.

[P072] Communication calls elicit selective neural responses in the inferior colliculus of the big brown bat (Eptesicus fuscus)

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Acoustic communication has been studied in many species, yet our understanding of the underlying neural mechanisms is incomplete. Bats are auditory specialists that use echoes from their own vocalizations to build a scene of their surroundings. At the same time, these social animals live in roosts and use their vocalizations to communicate with conspecifics. Important advances have been made in understanding the neural underpinnings of echolocation, but far less is known about the mechanisms supporting acoustic communication. In big brown bats (Eptesicus fuscus) call structure, along with behavioral context, appears to determine the function of acoustic signals. We aim to investigate the neural mechanisms that enable the big brown bat's discrimination of communication and echolocation calls from conspecifics, which differ in behavioral relevance but overlap in spectro-temporal features. Here we compare responses of single neurons in the Inferior colliculus (IC) to acoustic signals used by bats for spatial orientation and social communication. We recorded echolocation and communication signals of bats in flight and played back these sounds to awake, passively listening animals. We found that some neurons show selective responses to communication calls. Specifically, sequences of natural communication calls employed by bats as food claiming calls (FM Bouts) elicited strong responses in a subpopulation of neurons, while the same neurons showed weak or no responses to sequences of echolocation calls that matched the timing of the communication FM Bouts. STRF analysis was performed to further understand the stimulus features that contribute to the selectivity of these midbrain neural responses.

[P073] An efficient process in the cochlea for the echolocating bats to extract weak echo signals

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Echolocating bats extract a weak signal contained in an echo to avoid obstacles and to catch flying preys. Then, they need to eliminate interfering frequency components close to the signal frequency in their echo produced by conspecific bats and by environment. In the central auditory system a network may inhibit interfering frequency components by inhibitory synapses. However, in the cochlea mechanical suppressions on the basilar membrane may work well to reduce responses to noise components close to the signal carrier frequency in order to extract a weak signal. We examined the cochlear microphonics (CM) of echolocating FM bats (Eptesicus fuscus and Pipistrellus abramus) to investigate frequency specific suppressive activities in the cochlea. Our findings indicate strongly suppressed frequency ranges above and below their carrier frequency (the terminal frequency), which may eliminate conspecific interfering frequency components and environmental noise frequencies in the inner ear for extraction of weak signals carried by the terminal frequency. [Research supported by Grant-in-Aid for Scientific Research (B) 26280064 to H.R. from the MEXT of Japan and Shandong University Startup Research Grant to H.R.]

[P074] Spatiotemporal patterning of perceptual gaze by echolocating bats in densely cluttered scenes.

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During navigation, big brown bats (Eptesicus fuscus) emit sequences of biosonar pulses to perceive their immediate surroundings and guide flight. The temporal patterning and direction of these pulses are adapted by the bat in response to different acoustic environments. Interpulse intervals (IPIs) between pulses are adjusted to the scope of the scene – the overall scene depth and the proximity of the nearest objects. Broadcast durations are generally limited by the delay of echoes from the nearest objects to avoid pulse-echo overlap, but high clutter depth can cause the bat to extend these durations even when it means pulse-echo overlapping. Additionally, in open spaces the bat directs its broadcast beam towards the object of immediate interest by rotating its head, but in complex, cluttered surroundings the bat's beam instead follows its immediately upcoming flight path. To investigate these adaptive perceptual strategies, bats were flown in a flight room through an array of closely-spaced chains arranged into either a straight corridor or a sharp 90˚ left or right turn. Ultrasonic microphones located on the landing wall and directly above the turn in the chain array recorded the bats' biosonar pulses during flights. Analysis of biosonar pulses, especially of those emitted before turns and on failed flights, reveals both general strategies that bats use when faced with increasingly difficult navigational tasks and significant individual differences in how bats adapt their biosonar emissions. IPIs, durations, and beam orientation all reflect the bat's momentary focusing of attention to a specific direction and a specific distance.

Electropreception

[P075] Blind electric fish in a cave

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Eigenmannia is a genus of Gymnotiform fishes that uses autogenous electric fields in social communication and to detect nearby objects. These nocturnal fishes are found throughout the Amazon basin. Recently, a species of eyeless Eigenmannia were discovered in a cave in the Terra Ronca National park in Goais, Brazil. Using a grid of electrodes, we recorded the electric fields of these fish in their natural habitat. For comparison, we also used this system to record the electric fields of a species of Eigenmannia in a nearby stream. Using these data, we computed the 2D locations and movements within and around the grid. This allowed us to identify the frequencies of the electric fields of individual fish, the production of modulations of the electric field (social signals), and the positions of fish over time. The surface Eigenmannia showed a diurnal pattern of locomotor and social behavior, hiding in rock beds along the sides of the stream during daylight hours and emerging overnight, swimming over distances of at least several meters. During the day, these fish maintained nearly constant electric field frequencies, whereas at night these animals modulated their frequencies as described in previous studies. In contrast, we saw no evidence of diurnal modulation of behavior in the cave Eigenmannia. The cavefish produced similar categories of modulations but the electric field strengths of the cavefish were as strong or stronger than the surface fish. The cave Eigenmannia also appeared to show some territoriality in their swimming behavior whereas the surface Eigenmannia did not.

[P076] Phase-locking behavior of Adontosternarchus is controlled by amplitude information

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An apteronotid weakly electric fish, Adontosternarchus, emits stable and high-frequency electric organ discharges (700 to 1500 Hz). When encountered with an artificial signal of higher frequency than the fish's discharge, the fish raised its discharge frequency and matched it to that of the artificial signal resulting in phase locking where the timing of the fish's discharge was precisely stabilized at a particular phase of the artificial signal over a long period of time (up to minutes) with microsecond precision. Analyses of the phase-locking behaviors revealed that the phase values of the artificial stimulus at which the fish stabilizes the phase of its own discharge (called lock-in phases) have three populations between -180 and +180 degrees. During the frequency rise and the phase-locking behavior, the electrosensory system is exposed to the mixture of feedback signals from its electric organ discharges and the artificial signal. Since the signal mixture modulates both in amplitude and phase, we explored whether amplitude or phase information participated in driving the phase-locking behavior in two ways. (1) A numerical model that utilizes only amplitude information successfully predicted the three lock-in phase values. (2) Behavioral experiments, in which fish were deprived of phase information but exposed to model predicted amplitude modulations, demonstrated that phase-locking behaviors stabilized at the three lock-in phases were still observable without phase information. Phase information is represented in the midbrain of Adontosternarchus, it is not required for the phase-locking behaviour.

[P077] Effects of acute and chronic hypoxia exposure on active electric sensing in weakly electric fishes

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Aquatic hypoxia (low dissolved oxygen) occurs naturally in many habitats characterized by low light and low mixing. Hypoxia can severely limit fish performance, especially aerobically expensive behaviours, such as swimming. Very little is known about how hypoxia affects the acquisition of sensory information. Weakly electric fish offer an accessible proxy of sensory acquisition, their electric organ discharge (EOD), which can be easily monitored with electrodes in the water. These fish sense perturbations of their self-generated electric field caused by objects in their vicinity, such as prey. They also sense each other's EODs in the context of communication. In contrast to earlier estimates, the active electric sense appears to incur a sizable energetic cost with up to 30% or even 40% of routine metabolic rate being allocated to the generation of the EOD and the associated processing of electrosensory input. We therefore asked how different species of electric fish respond to acute and chronic exposure to hypoxia. Species producing a wave-type EOD responded with a severe reduction in EOD amplitude, which translates to a loss in range of electrolocation and -communication. Changes to EOD frequency were minimal, though. Mormyrid pulse-type fishes, on the other hand, lowered their rate of discharge, compromising temporal acuity of electrosensory input in order to maintain basic organismal functions. Long-term acclimation to hypoxia leads to increased tolerance to low-oxygen conditions and can partially recover aerobic performance of the animals, including swim performance and electric signalling.

[P078] Molecular mechanisms of sustained high-frequency firing in the electric organ cells of Eigenmannia virescens

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We investigated how the electric organ cells (electrocytes) of the electric fish Eigenmannia virescens unremittingly generate high-frequency action potentials (APs) at 200-600 Hz. The density and kinetic properties of potassium channels in the plasma membrane are key determinants of an excitable cell's functional capacity and the resulting metabolic cost. To better understand the role of potassium channels in allowing electrocytes to maintain constant high-frequency firing, we cloned the cDNAs encoding the Na+-activated K+ (KNa) channels that terminate the electrocyte AP. We identified two KNa channel subunits, eslack1 and eslick, closely related to KNa channels in other vertebrates, and a third novel KNa subunit, eslack2. All three are expressed on the anterior electrocyte membrane, more than 1 mm away from the voltage-gated Na+ channels expressed on the innervated posterior membrane. Whole-cell currents recorded from Xenopus laevis oocytes expressing these KNa channels revealed that eslick currents activate much more rapidly than eslack1 currents. Eigenmannia virescens individuals each exhibit a different tonic baseline frequency of the electric organ discharge (EOD). Using real-time quantitative PCR to determine mRNA levels of the three KNa channel genes, we found that expression levels of only eslick were positively correlated with EOD frequency. We also found that expression levels of the voltage-gated Na+ channel Nav1.4a, the inward rectifier K+ channel Kir6.2, and the Na+/K+ ATPase were positively correlated with EOD frequency. These results suggest that electrocyte ion channel expression patterns are tuned to the cell's firing rate, with implications for the maintenance of high firing rates in excitable cells such as central neurons.

[P079] Spatial distribution and electric organ discharge rate variations in the sand-dwelling fish Gymnorhamphichthys rondoni in a stream in Amazonas, Colombia

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Weakly electric fish can be affected by natural fluctuations in water conductivity for communication and navigation.Over one year and under natural conditions, we investigated the effect of water conductivity on the daily spatial distribution at rest and on the electric organ discharges (EOD) produced over 24 hours of the sand-dwelling fish Gymnorhamphichthys rondoni in a terra firme stream near Leticia (Amazonas). We sampled daily for the presence of fish inside a grid net (8.0 x 15.0 m divided into 120 quadrants) to determine their spatial distribution. Homemade electrodes connected to sound mini-amplifiers and MP3 digital recorders were used to record the EOD produced by fish over four consecutive days during six field trips every two months from July 2016 to July 2017. Water conductivity ranged from 13 to 58 µS/cm2 depending on rainfall and water level. G. rondoni showed a clumped spatial distribution at rest within the grid and they never changed their location during the daytime. At night, the number of foraging hours depended on the water level of the stream, and fish showed homing behavior after foraging. EOD at rest were maintained at 15 Hz, but random increases and interruptions can be detected. We suggest that clumping in spatial distribution and the number of foraging hours could be related to resources distribution and/or abiotic conditions, while increases in EOD can be related to random disturbances, and interruptions to inter- and intraspecific interactions. No clear effect of water conductivity was observed on the spatial distribution at rest or on the EOD in G. rondoni.

[P080] Generation of electric charge and electroreception in bumblebees

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Bumblebees carry electric charge. Almost always positive, this charge facilitates pollen transfer between bumblebee and flower during pollination and seems to play a role in the detection of electric fields. Despite the potential general impact of electric charge on pollination, very little is known about how bumblebees undergo charging. Bumblebees are positioned high on the positive end of the triboelectric series, hence they will readily lose electrons on contact or friction with other materials, including air. Whether their charging is solely due to their tribology or is also a response to abiotic conditions is investigated here. A novel method of measuring the electric charge on bees is presented that can be adapted to other insects. Bumblebee charge is shown to be affected by environmental conditions, such as humidity, the local electric field and the abundance and polarity of ions in the air. Local electric fields are also determined by the atmospheric potential gradient, which can vary greatly with weather conditions. Although bumblebees can discriminate between small electric fields in the lab, our results indicate that foraging activity does not change in response to strong electric fields simulating those found in stormy weather. In effect, stable or fluctuating strong electric fields appear not to interfere with bumblebee foraging.

[P081] The role of electro-communication in groups of mormyrid weakly electric fish investigated by introducing an electro-communicating dummy fish

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Weakly electric mormyrid fish emit brief, pulse-type electric organ discharges (EODs), which are used for active electrolocation and electro-communication. The African mormyrid Mormyrus rume proboscirostris can be used as a model system for studying social behaviour in captivity. In our study, we aimed at finding answers about the function of electro-communication for group organization of freely swimming M. rume. In particular, we wanted to know whether the structure of groups and the behaviour of their members persisted, if one fish was replaced by an artificial dummy fish emitting electrical playbacks. The locomotor and electric signalling behaviour in groups of three fish were recorded while they left a shelter and swam into an exposed area of their aquarium. Swimming trajectories, individual temporal discharge patterns and interactive electro-communication were analysed. Subsequently, the ahead swimming fish of each group was replaced by a robotic dummy that mimicked the electrical behaviour of the substituted individual. The behaviour of this mixed group and their electrical signalling patterns were compared to natural groups. Fish in mixed groups showed a more frequent following behaviour towards the dummy than fish in natural groups towards each other. Group cohesion in mixed groups was stronger than in natural groups, indicated by shorter distances between group members. Individuals showed similar overall electric signalling behaviour as in natural groups. Interactive electro-communication patterns were even more abundant towards the dummy than to a real fish swimming ahead, presumably because the dummy could not interact dynamically with the fish.

[P082] Mechanisms for reading out a latency code in the electrosensory system of mormyrid fish

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The precise timing of action potentials provides fast and accurate information about stimulus features in a number of sensory systems. Yet little is known regarding how such information is read out by downstream circuits. In the electrosensory system of mormyrid fish, a striking example of a latency code has been described, and the mechanisms for its central readout are accessible to study. Electroreceptors on the skin respond to object-induced distortions of fish's electric organ discharge (EOD) with tiny shifts in the latency of their first spike relative to the EOD. Behaviorally, fish have been shown to detect shifts as short as 0.1 ms. Numerous lines of evidence suggest that latency is read out in a prominent class of ELL interneurons, the granular cells (Grcs), via integration of electroreceptor input and precisely-timed motor corollary discharge (CD) input related to the fish's EOD. Here we use in vivo whole-cell methods to characterize electrosensory and CD responses in Grcs for the first time. Past models posit that CD inputs provide excitation which is steeply graded across the time window in which electroreceptor spikes shift. In contrast, we observed a CD-evoked inhibition followed by an extremely broad excitation. Nevertheless, we found (1) that shifts in electroreceptor spike latency induced by objects drove large changes in the amplitude of Grc responses without changing their timing and (2) that such responses depended on CD inputs. Ongoing experiments and modeling are aimed at developing a mechanistic model of latency readout in Grcs, including roles of additional circuit elements such as electrical synapses and rapid lateral inhibition.

[P083] Circadian and sex-linked differential expression of melanocortin receptor 5 and androgen receptor in the electric organ of Brachyhypopomus gauderio.

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South American nocturnal electric fish generate a continuous weak electric signal to navigate their environment in the dark, and communicate with other members of their social group. The electric signal of one species in particular, Brachyhypopomus gauderio, changes dynamically to optimize the trade-off between 1) the energetic costs of signal production, and 2) the signal's communication demands associated with deterring competitors and attracting mates. These electric signal's changes follow a circadian rhythm and differ between males and females. Previous work has identified the melanocortins and androgens as the main neuroendocrine substrates underlying B. gauderio's electric signal plasticity. To further characterize the endocrine pathways underlying electric signal plasticity, we isolated the complete sequence of the melanocortin receptor 5 (mc5r) gene, and the partial sequence of the androgen receptor (ar) gene from the electrogenic cells that composed the electric organ (known as electrocytes). We also characterized differences in gene expression of mc5r and ar from electrocytes

harvested from males and females during the day and night, to capture the sexual dimorphism and circadian rhythmicity of the electric signal. The results of this project will help determine whether changes in gene expression for both the mc5r and ar genes area associated with variations in electric signal. By establishing the connection between hormone receptors' gene expression and electric signal plasticity, we hope to provide further insight into the hormonal regulation of electrogenic cells.

[P084] How does motor activity affect the sensory information received by Gnathonemus petersii?

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Interaction between sensory and motor systems is crucial for understanding animal behaviour. Sensory systems help inform individuals on how they should behave in a given context, and egocentric motions can modulate available sensory information. To understand how these systems interact, we studied the weakly electric fish Gnathonemus petersii, which perceives nearby objects using active electrolocation. Individuals produce pulse type electric organ discharges (EODs) which form low voltage electric fields that are distorted by nearby objects. Skin receptors detect changes in field properties facilitating 'electrical images' of the environment. Unusually, weakly electric fishes can swim forwards and backwards. We tested whether there were differences in the electrical information gathered by G.petersii during forwards and backwards swimming. We recorded the swimming behaviour of three individuals in the presence and absence of objects whilst recording their EOD activity. Swimming sequences were matched with EODs. Time between EODs (IDIs) was calculated as a proxy for sensory activity. We found a significant difference in IDI duration between backwards and forwards swimming during object exploration only. Forwards swimming was found to have the smaller IDI mean in three out of four conditions. Our data suggest that both swimming modes are important for information gathering though forwards swimming may facilitate more sampling. However, object shape and visit number look to be important too. Current work is exploring how important body movement is for shape discrimination. Our results suggest that by modulating its movements in synchrony with sensory discharges, G.petersii can acquire additional information about its environment.

[P085] Rapid Evolution of a Voltage-gated Sodium Channel Gene in a lineage of Electric Fish Leads to a Persistent Sodium Current

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Most weakly electric fish navigate and communicate by sensing electric signals generated by their muscle-derived electric organs. Adults of one lineage (Apteronotidae, Gymnotiformes) which may discharge their electric organs in excess of 1 kHz, instead, have an electric organ derived from the axons of specialized spinal neurons (electromotorneurons; EMNs). EMNs fire spontaneously and are the fastest firing neurons known. This biophysically extreme phenotype depends upon a persistent sodium current, whose molecular underpinnings are unknown. We show that a skeletal muscle-specific sodium channel gene (scn4ab) duplicated in one group within this clade (the Apteronotini) and, within ~2 million years, one of the duplicate genes (scn4ab1) began expressing in the spinal cord, a novel site of expression for this gene. Concurrently, the channel gene evolved at a high rate under positive selection (dN/dS >1.0) resulting in a number of amino acid substitutions which were mainly in regions of the channel responsible for inactivation: the inactivation "gate" (the DIII-DIV intracellular loop) which binds to the inactivation "receptor" (DIV S4-S5 linker) in the inner mouth of the channel to block the pore. Because these regions are so highly conserved across vertebrates, we introduced these amino acid substitutions into the homologous regions of a human sodium channel (hNav1.5) to assess if they altered channel properties. As predicted, they initiated a persistent sodium current (~6% of peak current). Thus, a novel adaptation allowing extreme neuronal firing arose from a duplication, change in expression, and rapid (~2 million years) sequence evolution of a muscle-expressing sodium channel gene.

Somatosensory Processing

[P086] A balance of sensory activity is required for the correct development of the corpus callosum.

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Rodents navigate the world primarily by using somatosensory information conveyed by their facial whiskers. The whiskers are represented in a precise point-to-point topographically conserved map in the cerebral cortex, where responses from a single whisker can be isolated to a single “barrel” structure. We removed the whiskers from one side of the face during a developmental period, thereby depriving mice of somatosensory input unilaterally, and investigated the effects on interhemispheric connectivity. We found that unilateral deprivation resulted in disrupted formation of the corpus callosum, which connects the two hemispheres and allows them to communicate. Further, we isolated two critical periods of exuberant callosal development, and found that sensory deprivation primarily affects the later period, which coincides with axonal arborisation and synaptogenesis, rather than axon guidance. Interestingly, when we instead disrupted sensory input bilaterally, this phenotype was partially rescued. We additionally confirmed that this morphological rescue was recapitulated functionally by probing the circuit connectivity optogenetically. Finally, we found that activity also needs to be matched in a precise spatial pattern between the two hemispheres, by removing whiskers to the same extent on either sides in either symmetrical or asymmetrical patterns. Cumulatively, this work demonstrates that neuronal activity driven by somatosensory input needs to be balanced between the two hemispheres during a critical period of development for the correct development of the corpus callosum.

[P087] Re-discovering the cephalopod brains using modern technology

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Cephalopods have the most complicated central nervous system (CNS) of all invertebrates at both anatomical and functional levels. The pioneering neuroanatomical work of Cajal and Young made this clear decades or indeed a century ago. Here using the new technique of diffusion magnetic resonance imagery (dMRI) focused on the squid brain and validated with the same traditional Golgi techniques used by Cajal and Young, we shed new light on this remarkable structure. A new macroscale brain-wide neural tractography is erected with over 200 previously undescribed lobe interconnections and many novel within-lobe morphologies. As with vertebrate connectomes this ‘circuit diagram’ for the squid suggests a set of functional morphology interactions and leads to new predictions at the behavioural level. The brains of all cephalopod groups, octopus, cuttlefish, squid and nautilus, are built around a circum-oesophageal set of ganglia or lobes that have expanded dramatically along with the visual and limb-based tactile capability that sets cephalopods apart from other molluscs. These advances have allowed the astonishing feats of camouflage, colour change, manipulation and intelligence in the cognitive realm that cephalopods are capable of and that continue to fascinate both scientists and the public.

[P088] Interspecific differences in somatosensory abilities in waterfowl

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Waterfowl (Anseriformes) are made up of a wide variety of species including ducks, geese, and swans. Species within this group exploit a wide variety of food sources, with differences in feeding ecology likely linked to species specific beak adaptions. Despite wide variations in beak morphology, diet and foraging behavior, interspecific differences in somatosensory abilities in waterfowl and how this relates to different feeding strategies and prey type is largely unknown. We examined the location of beak mechanoreceptors using computer topography (CT) and the number of fibers in each of the branches of the trigeminal nerve in 30 species of waterfowl. We show that considerable variation exists in the morphology of the beak, location of mechanoreceptors and number of nerve fibers. Species like ruddy ducks (Oxyura jamaicensis) were characterized by considerable more nerve fibers than other ducks, especially those species that catch fish, like mergansers. Nerve fiber counts were a good indication of how much information is moving from the beak to the brain, and variation across species highlighted the variability in how reliant each species is on tactile information from the beak. Mechanoreceptor location was again highly variable across species, and again ruddy ducks had the most unusually distribution. Ruddy ducks are diving ducks that forage in murky water, and even forage at night. This foraging ecology likely requires an extreme reliance on tactile information, which could explain these anatomical differences. Overall, the differences found in the anatomy of waterfowl beaks matched each species specific foraging behavior and prey type.

[P089] Context- and state- dependent activation of a descending interneuron in the stick insect Carausius morosus.

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Stick insects actively move their antennae to scan their surroundings during walking. When an antenna touches an obstacle the animal often reacts with a rapid reaching movement of the front leg. An identified descending interneuron potentially involved in this behavior is the contralateral ON-type velocity-sensitive neuron (cONv), that connects the brain to the thoracic ganglia. cONv encodes both antennal joint-angle velocity and substrate vibration. In stationary animals, cONv has highly fluctuating spontaneous activity that may reach rates similar to those measured during antennal movement at moderate velocities. How can cONv reliably encode joint movement in the presence of such strongly fluctuating spontaneous activity and how does it respond depending on the behavioural state and stimulus history of the animal? To answer these questions, we recorded the bilateral pair of cONv and tracked antennal motion in stationary animals. Substrate vibration induced by taps onto the table were encoded with a single spike per tap. Taps delivered at the frequency of footfall during walking reduced spontaneous activity of both neurons. Antennal stimulation did so only in the neuron contralateral to the antenna that moved. Preliminary results suggest that the combination of both modalities reduces the spontaneous activity more than a single modality. Furthermore, we found no movement-related modulation of cONv activity during active exploration, suggesting a strong dependence on behavioural state. Preliminary results show that responses to interrupted active antennal movements resemble those to passive deflection, implying that cONv acts as a reliable antennal contact detector under behaviourally relevant conditions.

[P090] Soft senses: mechanosensing in the body wall of caterpillars

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The types of sensory information necessary to control movements in soft-bodied animals are largely unknown and difficult to study in aquatic and burrowing animals. However, the tobacco hornworm, Manduca sexta is a soft scansorial insect that is as an excellent model system for studying such sensory-motor integration. Because Manduca lacks image–forming eyes most sensory feedback for locomotion is mediated by mechanosensors including filiform hairs and campaniform sensilla on the body surface and scolopodial stretch receptors attached to internal tissues. In addition, the internal body wall is tiled by a plexus of multidendritic sensory neurons whose role in locomotion is poorly understood. Recordings from sensory nerves in the abdomen show that neurons associated with the body wall are sensitive to rapid thermal stimuli and to very small amplitude strains, including vibration. Using a novel semi-intact preparation that maintains hoop stress of the body wall while allowing controlled mechanical displacements and simultaneous recordings from specific nerves, we are characterizing the response properties of these mechanosensing neurons. One goal of these studies is to determine how mechanical feedback is collected, processed and incorporated into the control of movements by a deformable climbing animal. These findings will then be used to design better control systems for soft robots working in complex environments.

[P091] Vibrational communication in chameleons: Part II. Behavioral contexts for production of and responses to vibration signals

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Substrate-borne vibrations have been reported to be produced by the veiled chameleon (Chamaeleo calyptratus). We propose that these vibrations are produced via contraction of muscles surrounding the gular pouch (see Part I.). We first examined the behavioral responses of C. calyptratus, and the related C. gracilis, to vibrations by placing chameleons, one at a time, on a wooden dowel attached to a permanent magnetic shaker. We recorded each chameleon's behavior before, during, and after a three-pulse vibrational stimulus of 25, 50, 150, 300, or 600 Hz (acceleration of 6 mm/s²). Both species exhibited a stop-behavioral response (i.e. no movement) when exposed to a stimulus of 50 or 150 Hz, while displaying a reduced sensitivity at all other frequencies (i.e., less or no reduction in movement). A second set of experiments recorded chameleon behavior and vibration production in dominance (male-male), courtship (male-female), predator-prey (adult-juvenile), and heterospecific contexts. A pair of randomly selected chameleons was placed on a dowel in a wooden box with individuals being separated by clear Plexiglas. Initially, the Plexiglas was covered by an opaque sheet, which was removed half-way through the experiment. Behavior (via digital camera) and vibrations (via accelerometer) were recorded both before and after the chameleons could view each other. In the presence of conspecifics and heterospecifics, chameleons produced sinusoidal vibrations at dominant frequencies near 140 Hz. Vibration production was particularly strong when two males were placed together. These findings improve the understanding of communication between chameleons, and can be utilized as a basis for further research into their use of substrate-borne vibrations.

[P092] Vibrational communication in chameleons: Part I. Specializations for vibration production and detection

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Though less studied than acoustic communication, a number of animals communicate via substrate vibrations. One group that has been understudied in terms of vibratory communication is reptiles, likely because their vocalization abilities are often lacking and entire groups are deaf to airborne sounds. An exception is the veiled chameleon, Chamaeleo calyptratus, which can produce low-frequency vibrations emanating from the throat region, which we hypothesize to be produced by throat muscles and amplified by the gular pouch, a tracheal out-pocketing. We used electromyography to record the activity of muscles surrounding the gular pouch of C. calyptratus during vibration production. We found that the Mm. sternohyoideus superficialis et profundus, Mm. mandibulohyoideus, and M. levator scapulae were active during the production of biotremors. We hypothesize that chameleons, which do not hear well, detect these low-frequency vibrations through tactile sensation. Using scanning electron microscopy, we examined differences in skin specializations of the tail and plantar and dorsal surfaces of the feet of two species with a gular pouch, Chamaeleo calyptratus and C. gracilis, and one species lacking a gular pouch, Trioceros jacksonii. Tufts, groups of longer setae, were observed on the surface of skin of tail, plantar and dorsal sides of feet, and back, with these tufts differing between species in morphology and width. Preliminary results show that plantar foot setae are longer in species with a gular pouch compared to those lacking one, suggesting that longer setae may be useful in detecting low-frequency vibrations. This gular-pouch mechanism of vibration production represents a completely novel form of communication in a lizard.

Locomotion

[P093] The influence of vertical and lateral flying frequencies on odour tracking flying robot

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Insects are experts in odour tracking and use olfactory information for navigation a lot. They can find the sources, such as fruits and pheromones, in a long distance far away from themselves. When searching the location of odour source, they fly in some special patterns such as a spiral and a zigzag. This kind of behaviour can be considered as various plume tracking algorithms and insects may choose the most suitable one based on current condition. The horizontal and vertical tracks, generally, can be plotted as sine waves with respect to time but with different amplitudes, relative phases, and frequencies. In this study, we focused on the influence of relationship between these two frequencies on tracking performance. By using a mini quadcopter (size: 180 × 180 × 40 mm, weight: 73 g) with gas sensors (MiCS-5524, SGX Sensortech, Switzerland), we can measure the concentration changes of odour during flight and compare the results with different movement conditions. The results may make us more understand the flying behaviour of insect, enable us to design a proper algorithm for a flying odour navigation robot, and realise the three-dimensional (3D) odour tracking in the future.

[P094] Free flight behavior of antenna-ablated hawkmoths

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Equilibrium sense is essential for flying insects to stabilize their posture during flight. Hawkmoths can receive equilibrium sense from the Johnston's organ at the antennal base. The Johnston's organs sense the Coriolis force from vibrated antennae during flight, like as the function of the dipterans' halteres (gyroscopic organs originated from hindwings). And ablation of the antennae induces instability of flight behavior (backward flight and crash) due to the loss of equilibrium sense (Sane et al., Science, 2007). However, it is still unknown how the loss of equilibrium sense changes the body kinematics during free flight, because it has been difficult to capture the instable flight behaviors of antenna-ablated hawkmoths in a limited recording area of high-speed cameras. In this study, we tried to capture feeding behaviors of antenna-ablated hawkmoths (Agrius convolvuli) in front of an artificial flower (hovering or approaching at low speed). Surprisingly, not many but some antenna-ablated moths fully performed the feeding behaviors, and we successfully compared their body kinematics between before and after the treatment. The antenna-ablated moths increased wing beat frequency and decreased their body pitch angle, which seemed to be a compensatory response to avoid the backward flight induced by the ablation. Furthermore, we observed oscillatory head roll movements followed by body rolling during approaching flight. We speculated that the equilibrium sense from the Johnston's organ is used for head control for gaze stabilization, or the antenna-ablated moths tried to increase the input gain of the reduced equilibrium sense by actively moving the head.

[P095] The effect of gait variation on path integration in dung beetles

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Like other ball-rolling dung beetles, Scarabaeus galenus can move dung away in a straight line from the source to be dug down at an appropriate location. Unlike other ball-rolling dung beetles, however, S. galenus may also build burrows in which they stockpile dung through repeated journeys to a nearby food source. Even more unusual is that these beetles will change the gait used to transport the dung depending on the source. If the dung is small dried antelope pellets, the beetles walk backwards to the burrow while carrying a pellet in their two hind legs. If the dung is wet, the beetles form it into a ball and roll it backwards to the burrow using just their two front legs to push of the ground. The difference between these gaits have significant implications for the path-integrating system they use to find their burrow: The initial step-count is made while walking forwards to the food source but it is replayed on the way back to the burrow either while walking backwards taking steps with four legs, or taking steps with just two. We find that gait affects both stride length and the orientation and stability of the visual field that is used to read the celestial compass cues necessary for orienting towards the burrow with consequences for their navigation precision. Our findings have implications for understanding the mechanisms of path integration and understanding how animals compensate for disturbances, either external or internal when navigating.

[P097] The nervous control of ciliary locomotion in Gastropod Molluscs; neuroanatomy and physiology

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The nervous systems of molluscs have provided insights into neural function and neural circuitry since Hodgkin and Huxley's work on the squid giant axon. Indeed, opisthobranch and pulmonate gastropods have served as model systems in which neural systems for learning and memory, central pattern generators, and olfactory process have been investigated. Here we present research on neural mechanisms underlying motor control of crawling in both a nudibranch, Tritonia diomedea, and pulmonate, Lymnaea stagnalis. Most gastropod molluscs use their foot to locomote, despite differences in the substrate over which they crawl. Our research over the past years has focused on morphological and neural structures of the foot and how they promote locomotion. The pond snail, L. stagnalis, is a freshwater gastropod that employs both muscular and cilia during crawling, whereas T. diomedea is a marine nudibranch that uses cilia only to provide the propulsive force during crawling. Here we investigated the morphological characteristics foot tissue from both species as well as the distribution of neurotransmitters previously found to be involved in locomotory behavior. To determine the structure of the foot tissue we used histological methods to differentiate tissue and cell type distributions. In addition, we used immunohistological techniques to determine the distribution of putative neuro-active molecules in the foot tissue as well as the fused central ganglia. Our result indicate that there is much overlap in the neurotransmitters involved in controlling locomotion and that rearrangement of foot musculature and mucus secreting glands and cells differ substantially in the two species.

[P098] Contextual modulation of escape behavior by multisensory integration in the cricket Gryllus bimaculatus

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To survive from predators in various situations, escape performance of animals largely depends on the surrounding context. The context dependency is mediated by multisensory integration between "trigger stimulus" that directly releases the escape response and "context stimulus" that represents the contextual information. Here, we addressed what spatio-temporal relationships between the trigger- and context stimuli affects the escape behavior in the crickets. Cricket is a useful model for this study because their sensory systems detecting the both trigger- and context stimuli are two well-studied, one of which is cercal mechanosensory system and the other is auditory system. We found a auditory stimulus (10-kHz tone) modulated the wind-elicited escape response in its direction and threshold. These auditory modulations did not require the coincidence in azimuth of both stimuli, while the walking direction was modulated by sound not coinciding with but preceding to the airflow. This indicates that temporal relationship would be crucial for the context-dependent modulation of the escape. Electrophysiological recording of descending signals revealed that the neural responses to airflow were also affected by the sound that finished before the airflow onset, suggesting that the auditory contextual information was memorized in short term within the brain to modulate the wind-elicited behavior. Furthermore, we found that this auditory modulation was clearly induced by a high-frequency (15 kHz) but not by a low-frequency (5 kHz) sound. This result suggests that the crickets may perceive the high-frequency sound as an echo of bats that are predator of them and alter their escape behavior.

[P099] Dynamic gait transition in the centipede, Scolopendromorpha scolopocryptops rubiginosus

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Crawling using locomotory waves is a common method of locomotion for limbless and many-legged invertebrates. It is generally believed that the direction of locomotory waves is fixed for a given species. However, by recording and performing detailed analyses of the gait patterns of a centipede (Scolopendro- morpha scolopocryptops rubiginosus) in various situations, we find that it dynamically generates its gait to allow for locomotory waves that vary in direction according to the situations. By introducing an order parameter, the (wave index), to characterize locomotory waves, we show that gait patterns are associated with stride and wavenumber rather than rotation frequency.

[P100] Inhibitory reset supports fast locomotion in C. elegans

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An asymmetric inhibitory reset can increase the cycle frequency of motor circuits by promoting the end of each cycle. Such reset can be driven by a proprioceptive feedback, such as a limb arriving to a sub-maximal position, or by feedforward mechanism that terminates pacemaker activity. Integration and modulation of an inhibitory reset mechanism could provide a mechanism to increase the basal frequency and to fine tune the output. There is an asymmetric inhibitory set of synapses in the neuronal network that underlie locomotion behavior in the nematode C. elegans. This small network of 75 interconnected motoneurons coordinates forward and backward undulations. Almost all excitatory (cholinergic) motoneuron innervates an inhibitory (GABAergic) motoneuron as well as a neuromuscular junction. The inhibitory motoneurons, in turn, inhibit an antagonistic muscle, but only the ventral inhibitory neurons also inhibit ventral excitatory neurons. Could this set of synaptic connection underlie an inhibitory reset that regulate the rate of motor output?To address this question we combine experimental and computational approaches, including behavioral analysis, calcium imaging, genetic and transgenic manipulation and optogenetic, as well as a biomechanical model and simulation. We found that mutant animals, in which GABAergic transmission is absent undulate slower than wild type animals, and that similar slow undulatory behavior is induced by optogenetic inactivation of GABAergic neurons. We describe the coordinated activity of motoneurons and muscle cells during a locomotor cycle with calcium imaging. Finally, we demonstrate the contribution of a proprioceptive inhibitory reset to the robustness of fast locomotion in a biomechanical simulation.

[P101] Comparative morphology of motor control in a lineage of praying mantises

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Movement that is suited to an animal's environment and life style requires adaptations of both control systems (motor control circuits in the brain) and effectors (limbs). We report results from a comparative study of the morphology of these two in a diverse group of praying mantises (Mantodea: Tarachodidae). Mantises offer an opportunity to ask this question in a highly diverse set of species that share a crucial characteristic: they are all predators that use their forelegs to strike, grasp and hold their prey. This common task is accomplished by a variety of ecomorphs specialized for a substrate (grasses, soil, bark, etc.), hunting strategy (ambush vs pursuit), or mode of movement (climbing vs running). Using confocal and micro-CT imaging, we created 3D reconstructions of the major neuropils of the central complex (including the protocerebral bridge, fan-shaped body, ellipsoid body, and lateral accessory lobes). Similarly, we used micro-CT imaging to create 3D models of the grasping foreleg of the same set of species. Using a common set of geometric morphometric and phylogenetic comparative analyses for both structures, we identify features of the control system and effectors that are correlated with a particular lineage, or are potential examples of homoplasy between more distantly-related species adapted to a similar environment, hunting style, or mode of movement. Furthermore, analysis of the forelegs reveals reduced muscle volume and increased structural reinforcement of the cuticle in some species, associated with adaptations for crypsis independent of lineage. These results begin to describe an interaction between evolutionary history, the control of movement, and the physical form of these animals.

[P102] Aimed limb movements in a hemimetabolous insect are compensated for allometric wing growth

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For aimed limb movements to remain functional they must be adapted to developmental changes in body morphology and sensory-motor systems. Hemimetabolous development of the locust Schistocerca gregaria provides an opportunity to investigate such compensation. In adults, touching a wing leads to well described aimed movements of the ipsilateral hind limb that cross the target site. In juvenile animals the wings (wing buds) are not yet fully developed, and aimed scratching has not been investigated. When juveniles moult into adulthood, there is dramatic allometric growth and reorientation of the wings. The tips of the wings are located more posteriorly in adults than in juveniles. We show that: (1) both juvenile and adult locusts make scratching movements in which (2) the distal end of the tibia is aimed at different targets on the wings and abdomen. (3) Initial movement trajectories of scratches elicited by touch of the wing tip differ in juveniles and adults. (4) The cyclical components of scratches aimed at the wing tip also differ in juveniles and adults. And finally (5), hind leg joint angles at the point of closest approach to the wing tip target also differ in juveniles and adults, but for both, the angles at the closest point of approach to each target fall on a common continuum. We thus show that despite marked allometric growth and radical changes in wing morphology during locust development, scratches aimed at the wings remain targeted to appropriate locations, and the characteristic form of the movements remains the same.

[P103] Locomotor recovery after injury in the medicinal leech: novel proprioceptive pathways correlate with the return of locomotion

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We have shown previously that identified neurons in the brain are both necessary and sufficient for the activation and segmental coordination of crawling behavior in the medicinal leech. Thus, after brain removal (or transection

between the brain and segmental ganglia), leeches are refractory to crawling. After 10-14 days, however, they remarkably recover their ability to crawl. We have determined that nerve cords isolated from crawl-recovered leeches, and placed in a crawl-activating solution of dopamine, are incapable of fictive coordinated crawling. Although crawl-like bursting can be induced by dopamine, all crawl-specific coordination is lost (n = 18). These data have led us to study whether proprioceptors are able to substitute for the lost coordinating signals. Neurobiotin fills of identified stretch receptors, in post-injury crawl-recovered animals, show altered morphological projections—instead of projecting only to their home ganglion, they grow and project to adjacent ganglia, suggesting a role in segmental coordination. To test this idea, we prevented these proprioceptors from interacting with the anterior-most ganglion by cutting their nerve roots. In these animals (n = 3/7), no crawl recovery was observed after 1 month or longer. In a sub-set of these animals, we found that stretch receptors were able to regrow past their original nerve cut, innervating multiple ganglia. Leeches that had nerves severed, yet still recovered in under 1 month, showed afferent regrowth across the injury site with innervation of multiple ganglia (n =4/7). These results indicate that although stretch receptors play a limited role in oscillator coordination during crawling, they become a vital component during recovery-based crawl-network configuration.

[P104] Change in Electromyographic Patterns After Leg Amputation in the Cricket

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Insects exhibit flexible and resilient walking according to changes in their body properties, e.g. leg amputation. While control paradigm in coordinating leg movements, i.e. interlimb coordination, for such adaptive walking has been extensively discussed on past studies, e.g., based on behavioral observations and physiological experiments both in vitro and in vivo, the mechanism remains unknown. Understanding this mechanism is also useful for robotics fields, i.e., for establishing design principles of insect-like robots that can reproduce such ingenious behavior under the real world. To elucidate the adaptation mechanism after leg amputation in insects, we focused on electromyographic (EMG) patterns during locomotion, which represents activation patterns in the motor neurons innervated from the thoracic ganglia for adaptive leg coordination. Here, we examined this issue using the cricket (Gryllus bimaculatus), in which adaptive coordination in response to leg amputating patterns can be observed similar to other insects. To this end, we set a measurement system for a high-speed camera and EMG signals on a spherical-treadmill, where crickets walk freely before and after leg amputation. Simultaneous recordings of leg movements and multi-channel EMGs showed that muscle activation timings of protractor muscles in both middle legs tended to synchronize in phase when both legs were amputated at coxatrochanteral (CTr) joint. These results support the hypothesis that an intrinsic excitatory contralateral connection within mesothoracic ganglion exists and feedback from the mechanosensory information of the legs override the connection, resulting in anti-phase leg movements of a normal tripod gait.

[P106] Computational strategies underlying octopus arm coordination during naturalistic foraging

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The diversity evolution has made of the ancestral nervous system provides a rich source of alternative models for perception and decision making. The exceptional cognitive capacity of the octopus among this diversity makes them a particularly informative example of convergent intelligence. We investigated foraging behavior in the octopus to define the computational strategies they evolved to use against one of the principal selective pressures in the evolution of cognition: the need to localize and pursue food sources. Despite their visual acuity, octopuses are primarily nocturnal hunters, using the highly concentrated chemoreceptors in their suckers to detect and discriminate odors and can distinguish objects by their chemical composition alone. To study how the highly distributed octopus nervous system processes information under natural conditions we developed a foraging task for the octopus and combined this task with arm tracking algorithms. Data from these experiments suggest that octopuses use their arms to extract directional information from odor plumes and direct their movement toward the plume source. Expanding upon these findings, we employed new technology to characterize movement in three dimensions and define the algorithms octopuses use to collectively acquire, integrate and respond to information across the arms. Additionally, we used particle imaging velocimetry and fluid modeling to ascertain the dynamic forces acting on the arms during movement through water. Together, these results determine the information processing and control constraints on octopus sensorimotor feedback during naturalistic foraging. Understanding the highly distributed, embodied cognition of the octopus will motivate the development of novel strategies for solving problems involving complex, high dimensional information processing.

Motor Circuits

[P107] Interactions between the ventilation and spiracular motor patterns in the locust

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Insects exchange respiratory gases through an extensive network of tracheae that open to the surface of the body through segmental spiracles. In locusts, as in several other insect species, monitoring CO_2 release at rest or at low metabolic rates reveals a discontinuous gas exchange (DGE) pattern, consisting of short periods of spiracular gas flow, separated by long periods characterized by closure of the spiracular openings. In actively ventilating insects, such as the locust, respiration involves, in addition to spiracular activity, abdominal pumping movements. In order to better understand the complexity of insect respiratory motor activity and its neural control, we simultaneously monitored CO_2 emission, activity of the closer muscles of thoracic and abdominal spiracles, and the abdominal ventilatory muscles' motor patterns in fully intact desert locusts demonstrating DGE. Not as expected, we found that during the burst of CO_2 emission, the spiracles opened and closed rapidly rather than remained continuously open. This activity was strongly correlated with rhythmic abdominal ventilation activity. Abdominal spiracles were found to open out-of-phase of the thoracic ones and in-phase with the ventilation muscles activity, thus facilitating a unidirectional flow of air through the body. Bursts of abdominal constrictor muscles were tightly coupled to increased activity of the thoracic spiracle closure muscle also during the long periods of spiracle closure, facilitating efficient mixing of the tracheal gas content. Our reported data, supported by further in-vitro experiments, suggest strong coupling between the spiracular and the ventilation pattern generating circuits, and provide insights into their modulation and control by concentrations of respiratory gases.

[P108] Neurotransmitters and motoneuron contacts of multifunctional and behaviorally specialized turtle spinal cord interneurons

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The spinal cord can appropriately generate a variety of movements, even without brain input and movement-related sensory feedback, using a combination of multifunctional and behaviorally specialized interneurons. The adult turtle spinal cord can generate motor patterns for forward swimming, three forms of scratching, and limb withdrawal. We previously identified a morphological-physiological category of multifunctional spinal interneurons, transverse neurons (T neurons), that are strongly and rhythmically activated during swimming and scratching, and thus might be central pattern generator (CPG) neurons. We also previously described scratch-specialized neurons, activated during scratching but not swimming. How these types of interneurons affect downstream neurons is not yet known. Here, we used sharp microelectrodes to record intracellularly from spinal interneurons activated during these motor patterns in vivo and filled each with dyes. We retrogradely labeled motoneurons in advance via intraperitoneal injection of FluoroGold. We used immunocytochemistry of axon terminals of filled interneurons to identify their neurotransmitter(s) and possible synaptic contacts with motoneurons. We found that T neurons are heterogeneous with respect to their neurotransmitter, with some glutamatergic and others GABAergic or glycinergic. Thus, if T neurons are shared CPG components for swimming and scratching, then the CPG(s) may include both excitatory and inhibitory elements. We also found that at least some scratch-specialized neurons are glutamatergic; some have acetylcholine as a co-transmitter. Some scratch-specialized neurons directly contact motoneurons ipsilaterally. Thus, one possibility is that some scratch-specialized neurons directly excite motoneurons that need to be more strongly activated during scratching than during forward swimming, such as hip-flexor motoneurons.

[P110] Descending neuron control of Drosophila steering muscles during flight

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Small flies, such as Drosophila melanogaster, can execute elegant flight maneuvers with a remarkably sparse set of actuators. In flies, the power for flight is generated by a specialized set of asynchronous, stretch-activated muscles while control is orchestrated by only 12 muscles, each innervated by only a single motor neuron. These motor units can be further subdivided into two classes — phasic and tonic —according to their firing pattern. While tonic muscles continuously regulate fine-scale changes in wing motion, phasic muscles are recruited to execute large rapid maneuvers. Both of these classes require local mechanosensory feedback and multimodal input from the brain, conveyed by Descending Neurons (DNs). With a new set of genetic reagents, we were able to study a population of DNs projecting to the wing neuropil to investigate their roles in flight control. We optogenetically targeted small subsets of these DNs for activation, expressing CsChrimson in sparse split-GAL4 labeled DN lines during tethered flight, while directly recording from asynchronous, tonic, and phasic muscle, and simultaneously tracking wing kinematics. We specifically recorded the activity of the B1 tonic muscle and B2 phasic muscle, whose activities and associated effects on wing kinematics are well established. Ongoing studies indicate that while DN population coding mediates thrust and power, small changes in the activity of single pairs of DNs are sufficient to regulate the activity of B1 and B2. Furthermore, preliminary silencing experiments, using the inwardly-rectifying potassium channel, Kir2.1, indicate that these DNs may be instrumental in determining the firing phase of some phasic steering muscles.

[P111] Modulation of crustacean central pattern generators: Is the extent of modulation related to the need for flexibility in movement patterns?

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Most behaviors, including the rhythmic movements controlled by central pattern generators (CPGs), are flexible, enabling animals to alter movement patterns as internal and/or external conditions dictate. Although CPGs are hard-wired, they are multi-functional networks, facilitating the generation of flexibility: each network can generate multiple rhythmic outputs, due largely to the influence of locally and/or hormonally delivered neuromodulators. In the crustacean stomatogastric nervous system (STNS), which controls the movements of the foregut, one such CPG, the pyloric network, generates the triphasic pattern that controls the filtering apparatus of the foregut, i.e., pyloric filter. The STNS is highly modulated in most species that have been studied. However, in the kelp crab, Pugettia producta, whose diet is limited largely to kelp, many neuromodulators known to modulate activity in other crustaceans fail to alter the pyloric pattern. We hypothesized that the limited modulation in the Pugettia pyloric CPG is related to its limited diet. However, Pugettia is a majoid crab, and is thus not closely related to the Cancer crabs and lobsters in which the tested modulators elicit clear effects. To determine whether the decreased modulatory phenotype of the Pugettia STNS was more likely a function of diet or of phylogeny, we examined pyloric modulation in a closely related, but opportunistically feeding, majoid crab, Libinia emarginata. All of the modulators that failed to activate the Pugettia pyloric pattern were able to activate the pattern in Libinia, suggesting that the limited diet of Pugettia has led to a decrease in modulatory capacity in this species.

[P112] Behavioral variation correlates with differences in single neuron 5-HT receptor subtype expression within and across species

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Pleurobranchaea californica is a sea slug (Mollusca, Gastropoda) that produces a swimming behavior consisting of rhythmic body flexions. Individual Pleurobranchaea vary daily in whether they swim. This variation correlates with the ability of serotonin (5-HT) to enhance the strength of synapses made by a neuron in the swim central pattern generator called C2. Another species, Tritonia diomedea reliably produces the swimming behavior and does not exhibit the variation in serotonergic neuromodulation. A third species, Hermissenda crassicornis never produces this behavior and lacks the serotonergic neuromodulation of C2 synaptic strength. Here we found that expression of particular 5-HT receptor subtypes correlated with the production of swimming behavior.
Seven 5-HT receptor subtype genes were identified in whole-brain tissue. We isolated individual C2 neurons and performed both single neuron RNA sequencing and quantitative PCR to determine which receptors were expressed. We found that C2 neurons isolated from Pleurobranchaea that were swimming on the day of isolation expressed the 5-HT receptor subtypes 5-HT2a and 5-HT7 as did the Tritonia samples. These subtypes were absent from C2 isolated from Pleurobranchaea that did not swim on the day of testing and from Hermissenda C2 neurons. The expression of other G protein-coupled receptors did not correlate with the swimming behavior. The correlation between swimming and expression of specific 5-HT receptor subtypes indicates that these receptors may mediate the modulation of C2 synaptic strength and thus play an important role in swimming. Furthermore, the results suggest that rapid regulation of receptor expression could underlie daily changes in behavior.

[P113] Saliva of the Assassin Bug Platymeris bigguttatus (Reduviidae) rapidly abolishes prey nervous system escape-response without disrupting normal giant fiber activity.

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Many predators have evolved potent neurotoxins that quickly subdue their prey to prevent escape or harm to the predator. We have investigated generalist predatory assassin bugs, Platymeris bigguttatus (Hemiptera: Reduviidae), that inject neurotoxins and salivary enzymes through a slender beak into prey. Attacks on large prey, such as American cockroaches, Periplaneta americana, follow a stereotypic pattern of first briefly (4 min) biting the thorax, which immobilizes prey in 30sec. The bug then briefly (4min) bites the head. Then it feeds from the abdomen for scores of minutes. Immediately after prey is immobilized, it is unresponsive to wind puffs on the cerci, but electrophysiological recordings of the ventral nerve cord reveal typical activity in the giant fibers. When the legs stop moving, the jaws are fully adducted and are no longer able to bite a sucrose soaked probe, thus we are unsure of the purpose of the bite to the head. The mean mass of cockroach heads is less after the head bite than before, thus the bug may feed on the neural tissue. We “milk” the bugs, dry the saliva, and inject known concentrations into the cockroach body cavity to produce paralysis in 30 seconds. Bugs have principal and accessory salivary glands and injections reveal that only the posterior lobe of the principal gland produces neuroactive components. Legs are immobilized in tonus, and application of posterior lobe contents elicits increased firing of action potentials in elevator and depressor roots of the T1 ganglion. Chemical characterization is underway.

[P114] Neural differences underlying the rapid evolution of fly song

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The neural basis for behavioral evolution is poorly understood. Comparing homologous neurons across species can reveal how neural circuitry contributes to behavioural evolution, but homologous neurons cannot be identified and manipulated in most taxa. Here, we identify homologous courtship song neurons and compare their properties and functions by exporting neurogenetic reagents that label identified neurons in Drosophila melanogaster to D. yakuba. We found a conserved role for a cluster of brain neurons that establish a persistent courtship state. In contrast, a descending neuron with conserved electrophysiological properties drives different song types in each species. Our results suggest that song evolved, in part, due to differences in the neural circuitry downstream of this descending neuron. This experimental approach can be generalized to other identified neurons in D. melanogaster and therefore provides a novel experimental framework for studying how the nervous system has evolved to generate behavioral diversity.

[P115] Neural substrates for sensorimotor plasticity to control pecking with an experimentally extended bill in pigeons

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The ability to use tools as extended body parts has evolved not only in primates but also in birds. Primates' tool-use proved to be underpinned by the neural plasticity for sensorimotor control of body parts, called as 'body schema'. In contrast, neural substrates for sensorimotor control of extended body parts remains totally unknown in birds. Previously, we found the sensorimotor learning ability of pigeons to adapt pecking movement to the experimentally extended bill by the attachment of an artificial bill. The present study aimed to clarify telencephalic nuclei responsible for sensorimotor learning to control pecking with the extended bill. Neuronal activities following to pecking grains on the floor were measured immunohistochemically with brain-derived neurotorophic factor (BDNF) and c-fos proteins in several telencephalic nuclei of pigeons and compared among three groups; normal bill as control, 1-day bill-extension (1D), and 10-day bill-extension (10D). Candidate telencephalic nuclei included somatosensory and motor areas such as the hyperpallium, the nucleus basorostralis, the frontal nidopallium, and the arcopallium. Both BDNF and c-fos immuno-positive neurons in the hyperpallium were found more in the 1D group than in the others. Given the anatomical location of the hyperpallium to receive the somatosensory afferents and to send the motor efference, the present results suggest the role of the hyperpallium in sensorimotor plasticity to control extended body parts.

[P116] Modular organisation of prey capture behaviour in zebrafish larvae

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Zebrafish larvae use their visual system to hunt prey items such as paramecia, which they must localise, pursue and capture within a three-dimensional environment. During the pursuit phase of hunting behaviour, larvae converge their eyes and perform a series of low-amplitude orienting swims that bring the prey within striking distance. Previously, our lab described the features of the visual stimulus driving these orienting movements and identified the visual components of the underlying circuit. The motor circuits recruited during hunting, however, remain unknown. By studying the kinematics of the behaviour, our aim is to determine whether hunting swims are drawn from a pool of modular behavioural motifs or a continuous behavioural space, which we argue has implications for the underlying motor circuitry. We use high-speed recordings and computer vision to track the tail, jaw and eyes of freely swimming larvae hunting paramecia and apply unsupervised machine learning algorithms to identify potential kinematic motifs recurrent in the hunting behaviour. Our analysis suggests that larvae transition from graded swims during prey pursuit to more stereotyped motor programs during capture manoeuvres. We identify a stereotyped jaw movement present during the capture phase of the behaviour that larvae combine with one of two kinematically distinct capture strikes, which we term the "forward strike" and the "s-strike". Based on these findings, we hypothesise that pursuit swims are generated by distributed premotor circuits while capture strikes are controlled by two smaller populations of command-like neurons, which we will now test using the genetic toolkit available for the zebrafish larva.

[P117] A second complete connectome: the larval CNS of the ascidian Ciona intestinalis

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The comprehensive compilation of synaptic circuits densely reconstructed in model brains is now both technically feasible and scientifically enabling, much as 30 years ago genomics was in molecular biology. Circuit information from EM will eventually be required to understand how differences between brains underlie differences in behaviour, or identify common synaptic circuits in different species. The diminutive brains of invertebrates are Nature's bounty for connectomic analyses. Pride of place goes to C. elegans and Drosophila also supports powerful genetic methods and interesting behaviours, but its neurons branch too profusely and require special methods to reconstruct at EM level. We have used serial-section EM to compile a complete densely reconstructed connectome for the CNS of an alternative simple brain more closely related to vertebrates, the tadpole larva of the tunicate Ciona intestinalis. The dorsal, tubular CNS of the ~1-mm tadpole larva of this sibling vertebrate has ~330 cells distributed in a brain vesicle, motor ganglion and caudal nerve cord. Neurons are simple unipolar tubes with a single dendrite and few synapses. In one larva (Ryan et al., 2016, eLife) 177 neurons formed 6618 CNS synapses including 1772 neuromuscular junctions, augmented by 1206 gap junctions. Some synapses are bidirectional, others form reciprocal or serial motifs; 922 are polyadic. Axo-axonal synapses predominate. Most neurons have ciliary organelles. Some neurons show homology with vertebrate neurons, and have similar circuits. I will discuss the value of this new connectome, and how knowledge of it can open this tiny new model invertebrate brain to future neurobiological study in a basal chordate. Support: NSERC (Ottawa).

[P118] Unveiling the role of ovipositor extrusion in D.melanogaster during courtship

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Courtship behaviours allow animals to interact and assess their qualities before they commit to reproduction. Understanding the communication between male and female during courtship is crucial to describe the courtship process. Very little is known about female accepting and rejecting actions. Therefore, we set out to identify lines that display a female motor output in response to courtship. For that, we performed an activation screening in a collection of flies where only one or few descending brain neurons were labelled. We identified a line that labels two pairs of neurons that induce ovipositor extrusion (OE) when activated. OE is an action that happens exclusively during courtship: females extrude the ovipositor in response to male courtship. However, how ovipositor extrusion contributes to the progression of courtship is unclear because it is a low frequency event and both receptive (virgin) and unreceptive (mated) females do it. To elucidate the valence of OE, we recorded pairs of flies in a set up that allows us to analyse in detail different aspects of male courtship and the female responses to it. In addition to unmanipulated pairs, we recorded flies that would extrude the ovipositor whenever the male approached using optogenetics in a closed loop experiment. We are currently finishing the analysis. To assist our analysis we developed a method to automatically detect OE events to get a reliable quantification of the behaviour in a high throughput way.

[P119] Fast movements in soft-bodied caterpillars

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Fast, high-power movements in articulated living systems are usually driven by the rapid release of mechanical energy that is stored in stiff elastic structures by sustained muscle activation. Although soft animals lack stiff structures that are necessary for powerful movements, they can pressurize themselves and quickly displace fluids or deform constant volume tissues. Soft insect larvae are pressure-limited and not constant volume and yet can perform rapid movements. We are studying strike responses in Manduca sexta caterpillars to learn how soft animals control fast movements.
Caterpillars strike defensively at noxious stimuli by whipping their head backwards. To evoke strikes, we used infra-red lasers that provided localized and repeatable heat stimuli. They follow a broad general trajectory and target the stimulus site by a course correction towards the end of the strike. We have quantified the kinematics and dynamics of this behavior and have found evidence of generalized sensitization in the responses. Strikes can also be evoked with two successive sub-threshold stimuli at different locations. The tendency to strike decreases with increasing time intervals between the sub-threshold stimuli revealing a decay in sensitization.
Computational models of the caterpillar as a cylinder with sites that act as springs and bend upon load have shown that different arrangements of their stiffness and geometrical displacements render different bending patterns. We propose that dynamic regulation of stiffness across a soft structure is responsible for control. Caterpillars may be activating and de-activating muscle groups to create a "dynamic skeleton" to achieve targeting. We are exploring this hypothesis by detailed electromyography recordings.

[P120] Wing Motor Control in Drosophila

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To successfully reproduce, male fruit flies need to persuade females that they are good quality mating partners. Males accomplish this by singing a courtship song, a vital element of their courtship display, by extending a wing and vibrating it in a stereotyped manner. This innate, sex-specific acoustic signal provides the female with information for inter-species recognition and intra-species male quality assessment. Of course flies also use their wings to fly, and so shared neuromuscular components are employed during both behaviours. Our research explores the neural basis of courtship song generation, particularly at the underlying motor program and components involved. We aim to understand how multifunctionality is achieved in this sparse motor system and how neuromodulation may play a role in shaping these behaviours. We have performed functional imaging of the flight control muscles and discovered distinct muscle activity patterns during song compared to flight. We have established a comprehensive collection of genetic driver lines expressing in specific wing muscle motor neurons. By manipulation we examined their role during song production and uncovered that loss of single motor neuron function can lead to a complete loss of song or disrupt certain features of the song. Additionally, certain motor neurons which are critical for flight performance are not employed during song. Initial experiments altering octopaminergic neuronal output revealed that this neuromodulator may be implicated in the separation and selective stabilization of Drosophila wing motor patterns. We have gained novel insights into wing motor control and so propose a new model of muscle and motor neuron function during song production.

[P121] Homologous neurons serve as CPG members in one species and extrinsic neuromodulatory neurons in another species

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Homologous swimming behaviors in two nudibranch species (Melibe leonina and Dendronotus iris) are produced by homologous neurons with different synaptic connectivity. Here we show that a neuron that is a member of the swim central pattern generator (CPG) in Melibe serves as an extrinsic neuromodulator of the swim CPG in Dendronotus. In Melibe, the interneuron Si1 is rhythmically active in time with the swim motor pattern due to synaptic and electrical connections with other swim CPG neurons, making it a member of the swim CPG. In contrast, the Dendronotus Si1 is not rhythmically active but fires tonically during the swim motor pattern, indicating that it is not a swim CPG neuron. In this study, we found that stimulation of the Dendronotus Si1 could initiate or accelerate the swim motor pattern through bilateral excitatory synaptic actions onto other swim interneurons. Furthermore, it has a neuromodulatory action, potentiating the strength of excitatory synapses within the CPG. Dynamic clamp experiments demonstrated that the synaptic enhancement is necessary for the Dendronotus swim CPG to produce the swim motor pattern and that it shortens the burst period of the ongoing motor pattern. Thus, although the rhythmic swimming behaviors of Melibe and Dendronotus are homologous, there are important differences in the roles of homologous neurons; a neuron that functions as an intrinsic member of the CPG in one species serves as an extrinsic neuromodulator in the other species.

[P122] Modulation of the cardiac neuromuscular system of the American lobster, Homarus americanus, by differentially processed forms of the neuropeptide myosuppressin

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Peptides are important signaling molecules that enable flexibility in neuronal systems. The cardiac neuromuscular system of the American lobster, driven by a relatively simple pattern generator, the cardiac ganglion (CG), is a model for peptide modulation. Myosuppressin, an endogenous neuropeptide that is subject to two post-translational modifications, the cyclization of the (N)-terminal glutamine and the (C)-terminal amidation, acts on the CG to decrease contraction frequency. Actions at the muscle/neuromuscular junction contribute to a delayed increase in contraction amplitude. Here, we asked whether the presence of the post-translational modifications alters the response to the peptide, potentially adding another layer of flexibility to the system. In isolated whole hearts, mature myosuppressin and non-cyclized myosuppressin caused a decrease in contraction amplitude and frequency, followed by a large increase in contraction amplitude. The non-amidated isoform elicited a much smaller decrease in contraction amplitude and frequency, and no increase in amplitude. To determine whether the peptides exerted modulatory effects at the periphery, motor neurons were removed, and a motor nerve was electrically stimulated to elicit contractions. The mature and non-cyclized isoforms caused an increase in contraction amplitude, while the non-amidated isoform caused no significant change. On the isolated CG, all three isoforms decreased burst frequency and increased burst duration, as well as hyperpolarizing the membrane potential. Bioinformatic analyses predicted the presence of multiple myosuppressin receptors, which may be differentially distributed in the cardiac neuromuscular system. Selective binding to these receptors may be responsible for the functionality of all three peptides centrally, but not peripherally.

[P123] Female Drosophila respond to ejaculate with acoustic signals during copulation

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Male Drosophila melanogaster sing elaborate wing songs during courtship. Genes and neuronal circuits underlying this sex specific behaviour have been extensively studied. Females do not sing during courtship, presumably because they lack critical neuronal components. We recently discovered, however, that females produce highly structured sounds during copulation by vibrating both wings. Female copulation sounds are actively produced by several identified wing motor neurons. Furthermore, they depend on interneurons expressing the sex determination factor Doublesex. We find indication that female sound production is correlated with male ejaculate quality and/or quality. Males depleted of ejaculate by multiple recent matings, very old, very young or small males, all of which have less seminal fluid proteins, elicit fewer sounds. Sperm and products of the male accessory gland main cells are not necessary for sound production, but manipulation of accessory gland secondary cells leads to less sounds. Sounds might impact the behaviour of the copulating male and/or conspecific bystanders. We do not see any obvious effect of copulation sounds on copulation duration and fertility of the mating. When non-copulating males are exposed to female copulation sounds, they increase locomotion. This effect is similar to the one observed for male courtship song. Currently, our efforts are directed at identifying the sensory stimulus detected by the female and sensory neurons involved in this. We are also aiming at elucidating the function, behavioural relevance and adaptive value of this novel candidate signal.

[P124] Identifying the neuronal and genetic basis of sex-specific vocal behaviors

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Sexually dimorphic circuits provide a framework to study the role of hormones in establishing behavioral diversity. The vocal behaviors of the African clawed frog, Xenopus laevis, offer a unique opportunity to study sexually dimorphic behavior. Xenopus produce a wide repertoire of vocalizations, which consist of sound pulses resulting from contractions of the larynx. The mating vocalizations of male and female X. laevis are dimorphic both in rate and pattern: females produce monophasic calls with slow pulse frequencies, while male advertisement calls are biphasic with faster pulse frequencies. The circuit responsible for these vocalizations is a central pattern generator that includes a premotor nucleus and a motor nucleus that are sensitive to circulating androgens. Testosterone- or dihydrotestosterone-treated female X. laevis develop the capacity for male-like call production. We utilized comparative electrophysiological and transcriptomic approaches to characterize differences in function and gene expression in the premotor nucleus of males, females, and testosterone-treated females. Our data showed the development of male-typical electrophysiological properties in premotor vocal cells in testosterone-treated females after 8 weeks. RNAseq data further elucidate the cellular and genetic mechanisms underlying this dimorphism. This integrated approach allows us to identify not only the circuit dimorphisms that control sex-specific behaviors, but also the genetic basis of these circuit differences, leading to a more complete understanding of the mechanisms governing distinct behaviors.

Miscellaneous

[P125] Latching mechanisms to generate ultrafast movement of the trap jaw in the ant Odontomachus kuroiwae

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Virtually most animals have evolved variety of strategies to escape from threatening. Fast movement is one of the crucial behaviors to escape from predators. On the other hand, predators evolved faster movement to capture preys. Japanese trap jaw ant Odontomachus kuroiwae has pair of long mandibles that are quickly closed to capture a prey and to perform escape jump from potential threaten. The body mass of the ant is about 12 mg. The ant spread the mandible about 180 degrees and lock the mandible to detect a prey or threaten by the sensory hair on the mandible. Once the ant detects a prey, she closes the mandible at an ultra-high speed. The sequence of an escape jump is almost same. She hits the ground or a predator with the tip of the mandible and jumps away by using the reaction force from it. Latching the trap jaw is the first step to power the ultrafast movement. We here analyze 3-dimensional anatomical structure of the musculoskeletal system of the ant by using an X-ray micro-computed tomography (micro-CT). The micro-CT imaging allowed us to identify the fine anatomical structures of the mandibular joint and the mandibular muscles. We also performed simultaneous recording of the electromyography and high-speed imaging of the mandible movement. Kinematic analysis demonstrated how the ant latches the trap jaw to perform the ultrahigh fast movement.

[P126] Physiological differences underlying different defensive behaviors

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To avoid harm and to seek valuable resources are crucial behaviors for the survival of organisms. In vertebrates, it is well known that defensive behaviors are paralleled by physiological adaptations such as changes in heart rate, blood pressure or respiration that relates to the arousal state of the animal. Similarly to all animals studied, fruit flies exposed to looming stimuli [expanding shadow that mimics predator/object on collision course] react with different defensive behaviors (categorized in fight-flight and freezing). Here, we address a key question in neurobehavioral research: What are the differences in the physiological response underlying the different defensive behaviors? We image heart cardiomyocites of flies moving on a ball during looming stimulation. The heart of the flies alternates between two kinds of beatings -backward and forward - that push the hemolymph in two opposite directions - posterograde and anterograde, respectively. We observe that threat detection increases the proportional time of posterograde beating. Nevertheless, the strategy by which flies modulate the total length in each kind of beating differs between running and freezing. During running, the heart alternates very frequently between backward and forward beatings, whereas this alternation notably decreases during freezing. In addition, we observe that posterograde beat frequency increases during looming stimulation, but anterograde heart beat frequency is modulated deferentially depending on the defensive behavior. We see an increase on anterograde beat frequency when the fly is running and a decrease when it freezes. Thus, we show that each defensive behavior is accompanied by different heart responses, probably according to the particular physiological needs.

[P127] Predicting bumblebee trajectories during learning flight

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How animals structure their motion to memorize their home location efficiently and to return successfully to it is a fundamental question in biology. Bumblebees, for instance, must carefully learn the entrance of their nest before foraging large distances away from it in their quest for food. Although the learning flights of bumblebees have been systematically described during the last decades, little is known about how their flight is spatially and temporally organized. The departure flights of naive bumblebees may be controlled by an internal (“pre-programmed’) behavioural routine, as the bees did not have the chance to see its environment before its first flight. However, bumblebees need to take visual information into account, for example to avoid obstacles and learn the surrounding of their nest. Thus, a maneuver performed by a bumblebee at a given time during its first learning flight can be the result of an internal motion program, visual information, planning, or physical constraints imposed by the flight apparatus of the bee and the environment. Here, we study the timescale at which inertia, past motion, and the visual information, affect bumblebee flight maneuvers. To reveal these timescales, we used linear and non-linear time-series prediction techniques on learning flight of naive bumblebees. Indeed, when the current visual information has been used for the planning of a series of maneuvers, the later maneuvers within the series can be predicted from the past motion of the bee.

[P128] No missed connections: elaboration of a decision-making center in Stomatopoda

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Since the seminal work of Nils Holmgren in the early 1900s, comparative neuroanatomy in arthropods has greatly contributed to the functional and phylogenetic understanding of neural circuits. One region of recent interest has been the central complex (CX), which in insects comprises four midline substructures: the protocerebral bridge (PB), the fan-shaped body (FB), the ellipsoid body (EB), and the paired noduli (NO). The FB and EB constitute the upper and lower components of the central body (CB). Sensory integration and action selection of arthropod behavior are commonly attributed to the CX. Studies in flies indicate that the EB plays a role in visually-driven behaviors such as visual memory, orientation, and path integration. Although insect and malacostracan crustacean CXs share developmental origins, the latter generally possess far less structural complexity, consisting of a small bilateral PB which sits upon a thin, spindle-shaped CB. Stomatopod CXs are striking exceptions to this pattern. Stomatopod crustaceans, commonly known as mantis shrimps, are renowned for their unique sensory systems and elaborate behaviors. They possess modular, well-developed PBs and CBs. Furthermore, stomatopods are the only crustaceans thus far described to possess noduli-like neuropils. Our recent data indicate that mantis shrimps possess an additional midline neuropil whose immunostaining pattern with anti-DC0 and anti-GAD strongly resembles that of the insect EB, suggesting that the CBs of other malacostracan crustaceans are better compared to the insect FB, rather than to the whole insect CB. Here we discuss preliminary neuroanatomical data on the neural circuitry of this novel neuropil and suggest some functional implications.

[P129] Homing in a watery world: path integration and landmark navigation in a mantis shrimp

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Stomatopods, better known as mantis shrimp, are crustaceans which commonly inhabit holes in benthic marine environments for use as burrows. Many stomatopod species forage at extended distances from these burrows before returning back to their homes, risking predation. Since many mantis shrimp are central place foragers living in structurally complex environments, we hypothesized that these animals use landmark navigation and/or path integration to navigate their benthic environments. To determine which mechanisms are employed, Neogonodactylus oerstedii were placed in circular arenas in a glass-roofed greenhouse, with their burrows submerged from view. Foraging paths in the presence and absence of a landmark adjacent to the burrow were recorded. We found that return trips in the presence of the landmark were more direct than trips in the landmark’s absence. However, the initial direction of the return trips were generally oriented towards the burrow regardless of the presence of the landmark. Further, in the absence of a landmark, path lengths home were similar to the beeline distance to the burrow before a search behavior was initiated. To determine if N. oerstedii employ path integration when returning to their burrows, animals were translocated along a platform to a new location in the arena before homeward paths were initiated. These translocated animals exhibited homeward paths oriented towards the position where the burrow should have been had they not been moved, rather than towards the actual location of the burrow in the arena. These results indicate that N. oerstedii use landmark navigation in parallel with a path integration system to return to their burrows.

[P130] Electrical Properties of Developing Flight Muscle

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During metamorphosis, Manduca sexta caterpillars rapidly change both their morphology and their behavior. Over the course of approximately three weeks, the animal remodels its soft body and slow muscles into a form that can accommodate flight. To accomplish this, most larval muscles degenerate completely in the final days of larval life, allowing myoblasts to migrate into these locations and form new adult muscles. One notable exception is the Dorsal Longitudinal Flight Muscles (DFMs) and their precursors. The larval precursor muscles to the DFMs degenerate only partially, leaving a scaffold for the development of the adult DFMs. We have performed a series of ablation experiments on the precursor muscles in late larvae to determine the role of the scaffold in DFM development. Although ablation of the larval precursors disrupted DFM fiber number, the mass and volume were similar to sham operated animals and moths were able to produce qualitatively normal flight. Using this information, we can treat the scaffold primarily as a reliable location of developing muscle. This approach allows us to identify, study, and manipulate the DFMs throughout metamorphosis. We are using the unique advantages of this system to characterize the electrical and mechanical properties of insect flight muscle during its development. In particular, we can test how the motor unit makes the transition from a slow, forceful larval muscle adapted to high strains into a fast, powerful, low strain adult muscle suitable for flight.

[P131] Identifying the Molecular Mechanisms of Tissue Metamorphosis

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Full metamorphosis is found in insects, fish, mollusks, crustaceans, cnidarians, echinoderms, and amphibians. In addition to remodeling their tissues these animals interact with different ecological niches at each stage by changing their behavior. This involves a closely coordinated change in neural connections and the tissues controlled by them. The molecular mechanisms underlying these significant changes in morphology and behavior are still not well understood but can be traced in detail for the developing flight muscles of holometabolous insects. In Manduca sexta caterpillars the slow moving body wall muscles in the thorax are replaced during the pupal stage by fast, powerful adult flight muscle. During the early pupal stages larval muscles degenerate and most adult muscles form de novo. One exception is the Dorsal Flight Muscle (DFM) that develops by fusion of adult muscle precursors with the remains of specific motor units DEL and DIO2. We are studying the cellular and molecular processes underlying this transformation by co-culturing neurons with metamorphosing tissues, by heterochronic transplantation and through transcriptome analysis of the tissues at different developmental stages. One goal of these studies is to understand the processes that control tissue differentiation so that muscles can be engineered for use in robotic applications.

[P132] What in your right mind would make you do that? Ancestry influences acute decisions

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Decisions between alternative behavioral tactics rely on the interactions of experiences across evolutionary, developmental, and acute time scales. Evolutionary history can influence decisions on an acute scale by selecting for the sensitivity of decisions to salient cues in commonly encountered environments. To investigate how individuals from different populations have diverged in behavioral strategies, we tested how Trinidadian guppies (Poecilia reticulata) adopt alternative mating strategies when faced with acute naturally relevant social contexts. Different populations of these fish historically experienced either high or low levels of predation threat. We reared individuals from both high- and low-predation populations in either the presence or absence of predator chemical cues. Fish displayed distinct behavioral strategies when placed in different social conditions, and these strategies depended on both population and rearing environment. Using phosphorylated ribosomes as a marker of neural activation, we found that both population of origin and rearing condition altered neural responses to social context in several nodes of the social decision-making network and also within the optic tectum. This novel approach provides evidence for how decision making may be biased due to local environmental pressures across time scales.

[P133] Mammalian brains of different sizes are made of glial cells of similar densities

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Glial cells are now considered as key players of the brain physiology and metabolism, rather than just "brain glue". Brain structure mass scales across mammalian species with varying numbers of non-neuronal cells of fairly constant densities, but neurons of highly variable densities. Here we aim to determine if the relatively constant densities apply to only one subtype of highly abundant non-neuronal cells, or whether cell densities are constant across species and structures for each glial cell subtype. We use immunocytochemistry to NeuN, Iba1, S100b and Olig2 to quantify the total and relative numbers of neurons, microglia, astrocytes and oligodendrocytes in free nuclei prepared with the isotropic fractionator from brain structures of 33 species of five mammalian clades (10 marsupials, 4 artiodactyls, 8 primates, 5 afrotherians and 6 carnivores). While neuronal densities vary between 1,000 and 1,000,000 cells/mg across structures and species, microglial, astroglial and oligodendroglial densities vary between 1,000 and 10,000 cells/mg, 2,000 and 60,000, and 2,000 and 80,000 cells/mg, respectively. Such variation is however not systematic with brain structure mass, such that structures of similar size are composed of similar numbers of microglial, astroglial and oligodendroglial cells across different species and clades. As a consequence of systematic variation in neuronal but not glial cell densities, the ratio between numbers of each glial cell subtype and neurons decreases universally with increasing neuronal density across structures and species. These findings indicate that the addition of glial cells to mammalian brains is governed by evolutionarily conserved developmental mechanisms.

[P135] Selection for aggression in the Siamese fighting fish changes the brain, not the gonads

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The Siamese fighting fish Betta splendens has been selected across several centuries for street fighting contests. This strong directional selection has resulted in “fighter” strains phenotypically different from wildtypes, suggesting pleiotropic effects of the process of artificial selection for high aggression. In fish, androgens of gonadal origin, prominently testosterone (T) and 11-ketotestosterone (11KT), have been shown to promote aggression and were predicted to be elevated in fighters. We confirmed that fighter males are notoriously more aggressive than wildtype males. However, basal and aggression-induced circulating levels of T and 11KT were similar between the two male types. Gonadal relative volume also did not differ. On the contrary, whole brain gene-expression profiling showed major differences in the genomic response to a mirror-induced challenge between strains. Fighters had mostly an upregulation of genes when exposed to the agonistic challenge while the opposite was true for wildtypes. GO terms enriched in fighters after exposure to the mirror challenge were all absent from the equivalent list for wild-types. Cluster analysis showed that genes responding to the aggressive challenge in fighters were not constitutively regulated, as differences in the expression of these genes between strains were absent in the control situation. Overall, the results show that long-term selection for aggression in this species has not acted at the gonadal level nor in genes regulating androgen secretion. Rather, selection has acted on the brain response to aggressive challenges, favoring the upregulation of genes and the activation of alternative genomic pathways.

[P136] Neuropeptides as potential modulators of the behavioral-stage transitions in the desert ant Cataglyphis noda

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The organization of insect societies is highly sophisticated and unique in the animal kingdom. Different behavioral phenotypes contributing to various tasks are either associated with different morphs, age or stages of individuals. Although age-related polyethism has been studied in various species of social insects, the intrinsic mechanisms controlling the diverse changes in behavior are largely unknown. Due to the marked behavioral-stage transition from interior workers performing tasks inside the dark nest to primarily visually guided foragers navigating on long and windy trips to search for food under bright sunlight, Cataglyphis desert ants provide an excellent neuroethological model to study the neuronal control of this remarkable behavioral plasticity. Recent studies in social Hymenoptera, including Cataglyphis ants, suggested neuropeptides as potential modulators leading to changes in behavior related to behavioral-stage transitions. However, comprehensive neuropeptidomic studies are still missing in Cataglyphis. We therefore characterized neuropeptides in the brain of C. noda by using direct matrix assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). To investigate the spatial distribution of neuropeptides across the ant brain, we applied immunohistochemistry and MALDI imaging MS. Among 29 neuropeptides identified by MALDI IMS, we revealed detailed information on the spatial distribution of tachykinin, corazonin, allatostatin-A and allatotropin. To identify candidate neuropeptides for manipulations, we further employ quantitative PCR to reveal stage-related mRNA levels of the neuropeptides. Supported by DFG,

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[P137] The Insect Brain Database - A multi-species platform for comparative insect neuroscience

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Gaining insights into fundamental brain function depends crucially on comparing species to distinguish shared concepts from species specific specializations. Insects have proven valuable models for deciphering neural processes underlying behavior, given that they possess highly sophisticated behaviors, but have comparatively simple brains. Although insects have been used as models for neuroscience for over a century, the information obtained from a wide range of species is most often difficult to locate and raw data is usually unavailable. For Drosophila this has been addressed by online resources that bundle information for this species. Yet similar attempts in other insects have been of limited success. We have therefore created the InsectBrainDatabase (IBD), an online repository for anatomical and physiological data from any insect species (www.insectbraindb.org). The database is suited to house both new and historic data, and allows cross-species and within-species search using a unique graphical search interface. At its core, the IBD is organized into neuronal cell types, each of which receives a 'profile page'. This page is embedded into a hierarchical neuropil structure, which links to all anatomical and functional information relevant for this cell type. Individual experiments are additionally linked to these neuron pages and comprise specific anatomical or physiological results. Prior to making a dataset public, the data can be uploaded in ‘private mode’, so that the IBD is useable not only for data deposition but also for data management. Overall, with the IBD we hope to have generated a tool that will aid comparative insect neuroscience by providing an integrated platform for interspecies comparison and permanent data deposition.

[P138] Ultra-small, transparent and genetically modifiable vertebrate brain in Danionella translucida

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Understanding how distributed neuronal circuits integrate sensory information and generate behavior is a central goal of neuroscience. Yet, studying neuronal networks at single-cell resolution across the entire adult brain has been very difficult in vertebrates due to their size and opacity. We address this challenge by introducing the fish Danionella translucida as a model organism to neuroscience. This teleost remains small and transparent even in adulthood, when neural circuits and behavior have matured. Despite having the smallest known adult vertebrate brain, it displays a rich set of complex behaviors, including courtship, shoaling, schooling and acoustic communication. To enable optical activity measurements and perturbations with genetically encoded tools, we established CRISPR/Cas9 genome editing and Tol2 transgenesis techniques. These features make Danionella translucida a promising model organism for the study of adult vertebrate brain function at single-cell resolution.

[P139] The backdoor into behaviour: manipulative parasites as a tool for elucidating behavioural mechanisms

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The absolute control parasites can demonstrate over their host’s behaviour cannot be understated. The range of behaviours created or altered in the unfortunate host is vast and they can manifest subtly or dramatically. Take for instance an infected rat seeking out a cat, or an infected fish lingering on the surface of the water enabling a bird to catch it. Aggression, fear, motivation, attraction are all host behaviours, to name a few, modified by parasites. To learn or understand anything, we naturally look to masters first as teachers. However, what we could learn from manipulative parasites about behaviour at a mechanistic level is relatively untapped. While neuroethology has made tremendous strides since its origins, it struggles with the increasing complexity of physiological systems and the study of behaviours not immediately associated with auditory or visual systems (especially in complex organisms). Manipulative parasites could essentially provide neuroethologists with a ‘back door’ into investigating complex behaviours in a natural setting. Elucidating the impact of a parasite’s manipulative effort on neuronal functioning could be invaluable. Integral to this viewpoint, we will also present new research on the mechanisms behind a suicidal behaviour induced by mermithid nematodes infecting different arthropod hosts. Specifically, we will show protein changes (via MALDI-TOF mass spectrometry) that occur in the brain of mermithid infected earwig (Forficula auricularia) and sandhopper (Talorchestia quoyana) prior to the induced suicide. Ultimately we hope to bring the manipulative parasite approach to the mechanistic investigation of behaviour to the forefront of neuroethology.

[P140] Differences in nonapeptide neurophenotypes between alternative male morphs in a cichlid fish

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Males of the Kribensis cichlid Pelvicachromis pulcher exist in one of four different morphs, commonly distinguished by differences in the colour of their opercula and belly. The two most common morphs, “red” and “yellow” will both breed monogamously, but red males show preference to haremic breeding, while yellows do not. It has been suggested that yellows may follow a "satellite" strategy, or serve as subordinate helpers to harem holding males. The two morphs show behavioural differences. Red males grow more slowly, but are more active than yellow males; they also tend to use more escalated aggressive behaviours than yellow males. Here we present analyses of male-male contest behaviour between the morphs, and experiments measuring female preference for colour morph and other potentially sexually selected characters. We also examine differences in nonapeptide expression in the preoptic area of the hypothalamus which show differences between morphs, as well as variation within morphs associated with individual variation in social behaviour.

[P141] Drosophila female senses nutritional states of her mating partner and modulate sperm storage accordingly

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Many polyandrous females use sperm preferentially from the male that offers food items, but its neural mechanism remains unknown. Here, we show Drosophila females in nutritional debt obtain sugars from mating and store more sperm from males of better nutrition. To investigate neural and molecular mechanisms underlying this process, we focused our analyses on the brain neurons that produce a neuropeptide Diuretic hormone 44 (Dh44-PI). This is because Dh44-PI adjusts the timing of sperm ejection and as a consequence sperm storage in females, and because it also functions as a brain sugar sensor. Our evidence suggests that both glycaemia and copulation control the secretory activity of Dh44-PI. Furthermore, when we render Dh44-PI insensitive to glycaemia, the female becomes unable to modulate sperm storage in response to the nutritional status of her mating partner. To explain these, we are now testing a hypothesis that the ejaculate-born sugars enter hemocoel and activate Dh44-PI to facilitate sperm storage. This study offers a mechanistic insight on how nuptial gifts can bias female’s sexual selection after mating.

[P142] The change in sensory ecology during the vertebrate water-to-land transition provided a selective advantage for the evolution of planning systems

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The relationship between sensory input and behavior can be conceived along a spectrum. On one end of the spectrum is “reactive behavior” — a simple and rapid transformation of the sensory input — and on the other end is “deliberative behavior” — a choice from many internally simulated sequences of actions and corresponding potential outcomes, referred to as planning. Our prior work on the computational visual ecology of the vertebrate invasion of land revealed that eyes took in a largely blurry world at short range in ancestral aquatic environments. Just prior to moving on to land, we have shown that vertebrate eyes tripled in size after morphological changes that suggest crocodile-like hunting with aerial vision of invertebrate land dwellers. The increase in eye size and switch to aerial vision extended visual range by a factor of 100. Due to the increase in visual range and environmental complexity, the move onto land may have provided a selective benefit to animals that evolved planning. We show the results of simulated predator-prey interactions while controlling planning depth that are designed to examine this hypothesis. These simulations aim to examine how the benefit of planning varies with sensory range, and how it varies with the complexity of terrestrial landscapes as measured by entropy.

[P143] Waiting for Whiskers: Comparative Morphology of the Trigeminal Canal and a Sensory Scenario for the Evolution of Mammalian Facial Muscle

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Facial muscles are fundamental to the daily mammalian experience of communication, feeding, and sensation. However, identifying when these soft-tissue structures appeared in the fossil record is a challenge. This study proposes the single infraorbital foramen as an osteological correlate for the presence of facial muscles that move the mystacial vibrissae. Reconstructions of microCT scans of the neurovascular canals hosting the maxillary and mandibular branches of the trigeminus (cranial nerve V) in a sample of fossil and modern taxa reveal an evolutionary transition from (1) an ancestral state showing trigeminal canals with short, laterally-directed branches along the length of the jaws; (2) a derived stem mammalian state showing extensive branching and concentration of nerve foramina at the end of the snout inferring localized tactile sensitivity; and (3) a derived crown mammalian state with reduction in the number of branches and foramina, with the appearance of a single infraorbital foramen for the maxillary branch of the trigeminus in therians. This configuration is suggested to reduce the mechanical stress on sensory nerves embedded within a whisker pad mobilized by facial muscles. The results of this study introduce a scenario where a potential driver for the evolution of mammalian facial musculature was itself an innovation in active tactile sensation. I explore this hypothesis within the broader context of understanding in vertebrate development and mammalian evolutionary history.

[P144] The effects of ultrasound neuromodulation on behaviorally relevant neuronal firing patterns

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Focused ultrasound (FUS) is an emerging neuromodulation technology capable of modulating neuronal activity noninvasively. Reported effects of FUS technology have varied widely with respect to the magnitude and direction of modulation (e.g., neuronal excitation vs. inhibition). Most reports have examined effects resulting from targeting mammalian cortical areas. These preparations present several complicating factors; perhaps most saliently, FUS has been shown to activate cochlear hair cells irrespective of the targeted region, causing indirect neuromodulation. To elucidate the technology’s basal effects, we applied FUS to a more tractable nervous system, the medicinal leech, where we targeted single, identified motoneurons. When the preparation was bathed in normal saline, we found that FUS repeatedly and reversibly inhibited motoneuronal activity via a mechanism we hypothesize to be attributed to the mechanical gating of hyperpolarizing ion channels. Intriguingly, our effects varied when we introduced pharmacological agents into the preparation bath, including dopamine and serotonin, known to induce crawling and swimming behavioral states, respectively, in this model system. Rather than induce net neuronal inhibition, FUS in conjunction with these agents facilitated fictive locomotor-like bursting. We observed that FUS application elicited bursting patterns when the preparation was bathed in saline containing subthreshold concentrations of chemical neuromodulators (e.g., 50 μM dopamine, a concentration too low to reliably elicit crawling under normal conditions). This work demonstrates that the effects of neuromodulation technologies are state-dependent, and that single neuronal outcomes can vary based on behaviorally-relevant conductance states.

[P145] Effect of group size on the stinging responsiveness of honeybees

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Honeybees defend their colony against large predators by recruiting a large number of individuals to fight off the intruder, thus this behavior is collective in essence. Guard bees at the hive entrance signal the threat by releasing the sting alarm pheromone, a complex blend of over 40 compounds, thus alerting nestmates to fly out of the colony and harass the would-be predator. Although guarding and propensity to sting clearly have genetic determinants, multiple experiments have revealed that the expression of these traits is ultimately controlled by a strong social feedback. The nature of this feedback, however, remains unknown. To begin to unravel this mystery, we observed the stinging behavior of honeybees placed either alone or in groups of 2, 5 or 10 in a small arena where they faced a rotating dummy perceived as threatening. Surprisingly, analysis of the data revealed a negative group effect, whereby individuals became less likely to respond to a given amount of alarm pheromone as group size increased. These results challenge previous studies that were reporting a positive group effect in response to the alarm pheromone. In these cases however, the readout was measured as an increase in metabolic rate, which may represent arousal but would not necessarily be followed by stinging. Thus, we believe that more thorough experiments are required to elucidate how honeybees achieve the complex balance of defending their colony without depleting it of too much of its workforce.

[P146] Vasotocinergic neuronal activation during the establishment of the dominance-subordinate status in a weakly pulse-type electric fish, Gymnotus omarorum

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Understanding the general rules that govern the neuroendocrine bases of social behavior is a challenging task. The brain areas that control social behavior are organized in a highly-conserved network, the social brain network (SBN). Each specific behavioral condition corresponds to a pattern of activation of the SBN, which can thus be different among phenotypes. The activity of the SBN is shaped by the action of hormones and nonapeptides such as vasotocin which is produced in the preoptic area (POA) of teleosts. Gymnotus omarorum is a sexually monomorphic and highly aggressive species that displays territorial aggression all year around. In G. omarorum, vasotocin modulates agonistic behavior in a status dependent manner. Our aim was to describe the neuronal activation of POA neurons during agonistic encounters in G. omarorum non-breeding males. For this purpose, we carried out male-male contests, and the POAs of dominant males were immunolabeled for both c-fos and vasotocin, using expected dominants that did not fight as controls. We found more c-fos positive cells in dominant males with respect to controls. We did not find correlations between vasotocin/c-fos labeling and the percentage of time in which males displayed locomotor activity. We confirmed in dyadic agonistic encounters of G. omarorum that vasotocin neurons of dominants are differentially activated between dominants and subordinates. This study is the first one to show in teleosts that vasotocin is involved in the initial establishment of dominance, which is likely achieved by the liberation of vasotocin from vasotocinergic neurons in the POA.

[P147] Androgens don't drive me crazy!

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Domesticated species exhibit a common set of physiological, behavioral and morphological changes as compared with wildtypes, including low intraspecific aggression and low stress-response. However, in a few species domestication was used to exuberate aggressiveness, allowing to separate the effects of artificial selection on aggression from other traits related with adaptation to captivity. In these species we predict (1) a reduction in the stress response as a result of adaptation to captive conditions; (2) an increase in aggression and in androgen levels as these hormones have been suggested to promote aggression. We tested this hypothesis in the Siamese fighting fish Betta splendens, a species that has undergone artificial selection for high aggression for more than six centuries across South East Asia. This strain has been selectively bred for fighting by discarding loser batches and allowing winner batches to breed, resulting in fighter strains that differ from wildtypes in various traits. Here we compared a strain of fighter males with field-caught wildtype males reproduced in the lab for three generations. We show that fighters are indeed more aggressive than wildtypes. However, androgen levels did not differ between fighters and wildtypes, suggesting that selection for aggression did not act on androgens. On the other hand, and as predicted, baseline and stress-challenged cortisol levels were generally lower in the fighter strain as compared with wildtypes. Low cortisol has been associated both with domestication and high aggression levels and thus the hypothesis that selection for aggression also acted on the stress-axis cannot be discarded.

[P149] Brain and behavioural evidence of social cognition in octopus

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The brain of octopus has about the same number of neurons as a rabbit and seven times more than a mouse. Many studies revealed the richness of cephalopods behavioural repertoires, like colour-blind signal communication, mate guarding, social cognition and observational learning arguably comparable to the abilities of higher vertebrates. These cognitive abilities could be associated with complex social skills. However, knowledge of cephalopods' social interactions and the underlying neural pathways is sparse. Here, using a new method of diffusion magnetic resonance imagery (dMRI) focused on a systematic comparison of the octopus brains (solitary versus paired-bond species inhabiting the same niche), we shed new light on gross neuroanatomy and the underlying complex neural network. The high resolution MRI post-reconstruction (16.4 T) uncovered a far more detailed view of the octopus brains (isotropic resolution 30 μm). The overall central nervous system (CNS) layout shares a high degree of similarity in both species, with an exception of the vertical lobe (VL, memory and high command centre). The VL of the paired-bond octopus contains 7 distinct subdivisions (lobulae) in contrast to the regular 5 lobulae in any known solitary reef octopus. The tractography from these 7-lobulae presents different wiring patterns where those projections to the basal lobe (high motor centre) interact with numerous local short neural connections, suggesting multiple signal integration steps. To our knowledge, this is the first neuroanatomical feature, the number of subdivisions and the corresponding wiring diagram, that seems linked to social cognition; this hypothesis will be further tested by behavioural experiments.

[P150] Size or light: what drives neural investment in bull ants?

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Several insects are active exclusively at night where the visual signal-to-noise ratio is low. Nocturnal insects have evolved optical adaptations that include developing large lenses and wide photoreceptors to capture more light. However, these adaptations are not sufficient to explain visually guided behaviour at night where light intensities are 6-8 orders of magnitude dimmer than those during the day. Hence, there must be additional neural adaptations to explain visual behaviour in dim light conditions. At the same time, the size of the brain is also constrained by the size of the animal. Here, we aimed to identify how the volume of functionally distinct brain regions change in two congeneric, polymorphic bull ant species, the diurnal Myrmecia gulosa and the nocturnal Myrmecia midas. We took advantage of the extreme intraspecific size difference (8-27mm body length) in both species to identify how body size affects brain region scaling. Using volumetric analyses, we estimated the volumes of the brain and individual brain neuropils. We found that there was clear differential investment in specific brain regions in the two species: the diurnal ants invested significantly more into the optic lobes; the nocturnal ants invested more into the antennal lobes and mushroom bodies. We also found that there is a positive allometric relationship between neuropil volume and body size in both species. These results suggest that both size and light availability are important for the scaling of functionally distinct brain regions.

[P151] Changes in behavior and in the retina of cavefishes

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Similar phenotypes often evolve repeatedly when independent lineages are exposed to similar ecological conditions. The repeated degeneration or loss of traits, regressive evolution, has long been of particular interest to biologists, because it highlights the importance of stabilizing selection for maintenance of adaptive traits. Obligate cave-dwelling animals are well-known examples of regressive evolution because many lineages have independently either lost their eyes altogether or possess functionally degenerate eyes and pigmentation. We examined the degeneration of the retina during development in the cavefish Astyanax mexicanus via immunohistochemistry and transmission electron microscopy. Astyanax cavefish derived from surface fish ancestors, probably around a 1 million years ago. Different cavefish populations are believed to have originated in parallel, and likely have evolved eye degeneration independently. Cave forms of Astyanax arose through multiple colonizations, each followed by short-range dispersals. The oldest cavefish populations have extreme troglomorphic phenotypic changes, such as complete loss of the visual system structures and significantly enhanced constructive morphologies such as lateral lines. Astyanax show no phototaxic behavior in the laboratory. To examine behavioral changes, we examined another cavefish group in the field. We examined the phototaxic behavior of members of the cyprinid genus Sinocyclocheilus, which is endemic to China. Sinocyclocheilus is largest cavefish genus in the world and the second speciose genus in China. Species that were extremely troglomorphic showed no phototaxic behavior, and species that lived on the mouth of the cave had a strong negative phototaxic behavior. Convergent evolution has led to cave adaptation in multiple occasions.

[P152] Animal-microbial symbiosis in neuroethology: a hologenomic approach to understanding tetrodotoxin toxicity in rough-skinned newts (Taricha granulosa)

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Rough-skinned newts (Taricha granulosa) are poisonous salamanders that possess high concentrations of tetrodotoxin (TTX), a potent neurotoxin that blocks voltage-gated sodium channel (VGSC) conductance in neurons and muscle cells. TTX toxicity is a critical trait for newt survival and fitness, and the presence of TTX in newts has a rippling effect throughout their ecological community. However, the biosynthetic origin of TTX in newts is unknown and controversial. Here, we present evidence that TTX is produced by symbiotic bacteria within the skin microbiome of newts. We isolated nearly 350 strains of bacteria from newt skin and surveyed candidate genera for TTX production. Through the application of high-resolution HILIC-MS/MS, we identified TTX production in strains from several genera of Proteobacteria. Furthermore, to understand the mechanisms of TTX resistance in newts, we investigated the molecular adaptations in the VGSCs of newts. We cloned and sequenced the TTX binding site, the S5-S6 pore loop regions, of all six VGSC genes present in this species and identified several mutations present in all six genes, indicating a remarkable parallel evolution of TTX resistance across the VGSC gene family. Taken together, our results indicate that TTX is derived from the skin microbiome in the extremely toxic rough-skinned newt and that multiple adaptations in VGSCs were required for the newt nervous system to adapt to TTX toxicity. Overall, this research contributes to a growing understanding that symbiotic microbes can affect the physiology of nervous systems, and that evolution by natural selection may target genetic variation across both host and symbiont genomes, collectively termed the 'hologenome'.

Other Sensory Systems

[153] Setting the clock: Light and temperature entrainment in Drosophila

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Since all organisms on earth are subject to predictable but drastic daily environmental changes caused by the earth's rotation, they have evolved circadian clock mechanisms that regulate changes in behaviour, physiology and metabolism to ensure they occur at certain times during each day allowing adaption to the organism's environment. The Drosophila clock comprises 75 pairs of neurons grouped into identifiable clusters that sub-serve different circadian functions. Each neuron has a molecular oscillator that switches itself on and then off every 24h forming the molecular basis of the circadian clock. However, in order to run in synch with the environment this clock is entrained by 'Zeitgebers', mainly light and temperature. Individual neurons function as cell autonomous clocks and neurons communicate with each other for synchronising these autonomous clocks

and for conveying circadian information to the rest of the brain and body. But the precise connections between individual clock neurons and the input and output regions are largely unknown.
To address this gap and capitalising on fly genetics and pharmacology, we use a combined electrophysiological and optogenetic approach to characterise inputs and connections within this highly manipulable and compact clock circuit. We could thus recently show that the Ionotropic Receptor 25a, acting in the chordotonal organs, is required for behavioural synchronisation to low-amplitude temperature cycles and that the newly discovered light input factor Quasimodo, by acting on ion channels and transporters, affects both daily and acute light effects and helps setting the neurons in a day and a night state.

[P154] Back to the light: selection on sensory systems in Drosophila melanogaster

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Species have evolved sensory systems to help them make sense of the world according to their environment/lifestyle. For example, species living in dim lighting conditions (or in the absence of light) have highly specialised eyes adapted to low light levels, or have much smaller or non-existent eyes. However, although comparisons between extant species provide crucial information about how sensory systems have evolved, we still do not know how selection acts on sensory systems with changing lighting conditions. To better understand how sensory systems have been selected in the dark, we studied 'Dark-flies', a population of Drosophila melanogaster reared in the dark since 1954. We compared the sizes of the visual and olfactory systems in the Dark-flies and compared with a control strain of flies (Oregon-R-S strain), and Dark-flies that had been reared in normal lighting conditions for 1 and 65 generations. We measured the size of visual (optic lobes) and olfactory (antennal lobes) primary centres as well as the size of the central brains. We found that the Dark-flies reared in the dark possess smaller brains and optic lobes compared to the Oregon flies, but larger antennal lobes. After being under normal lighting conditions for 65 generations, brains and sensory systems of Dark-flies tend to be similar of similar size to those in Oregon flies. For the first time, our study shows how the investment in olfaction and vision changes over time, with dark rearing conditions selecting for individuals with larger olfactory capabilities, and light selecting for increasing visual systems.

[P155] Phototactic tails: Evolution and molecular basis of dermal photoreception in sea snakes

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Non-visual photoreception, i.e. light detection not involved in image forming vision, is well known among marine invertebrates, but far fewer examples have been described among vertebrates. Olive sea snakes, Aipysurus laevis, are the only snakes reported to show non-visual negative phototaxis and this behaviour is likely mediated through dermal photoreception: resting snakes respond to local illumination of the skin by retracting their vulnerable tail-paddles under rock or reef overhangs. We sought to better characterise dermal photoreception in sea snakes using behavioural, genetic and anatomical analyses. Our behavioural tests on captive snakes showed that tail phototaxis is not restricted to olive sea snakes and is probably shared by a clade of seven Aipysurus species. Transcriptome sequencing identified the expression of two non-visual opsins and 20 genes involved in phototransduction in the skin of phototactic sea snakes. We also report on preliminary investigations of the ecological significance of negative phototaxis by quantifying the prevalence of tail injuries in phototactic and non-phototactic species. This fascinating sensory specialisation may be more widespread in vertebrates than previously thought, and might be especially important to elongate aquatic animals (e.g. hagfish, lamprey, aquatic salamanders) that also have vulnerable tail paddles that are vital for underwater locomotion and anatomically remote from the concentration of sensory organs on the head.

[P156] Naturalistic stimulation increases high frequency sensitivity in spider mechanosensory neurons

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Action potential production is a nonlinear process, so that linear measures of sensory coding, such as frequency response functions, depend on the type of input signal used for measurement. We compared action potentials signals produced by naturalistic versus Gaussian distributed random mechanical inputs to VS-3 mechanosensory neurons of the spider Cupiennius salei. Naturalistic signals were created from laser vibrometry recordings of locusts walking on a Sansevieria plant. Action potentials were recorded intracellularly while mechanical input amplitudes were adjusted to give similar firing rates for the two types of stimulation. We computed linear and quadratic frequency response functions and coherence functions from the mechanical input to action potentials output signals. Linear responses were well-fitted by a power-law filter, so we attempted to fit quadratic responses by two block-structured models consisting of a power-law filter and a static, second-order nonlinearity in alternate cascade orders. Naturalistic signals had relatively higher amplitudes at lower frequencies, but caused significant increases in neuronal sensitivity to higher frequencies. Quadratic models consisting of a linear filter followed by a static nonlinearity were weakly favored over the reverse order cascade. These data indicate that VS-3 neurons nonlinearly adjust their response dynamics to accommodate different input signal frequency distributions.

[P157] Sharing Transducers

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The protosensory cell theory claims that sensory receptor cells of different modalities evolved from a common "ancestor". Thus one would expect to find parts of the cellular machinery being shared by receptors of different modalities. Evidence is accumulating that opsins can sense more than light. In Drosophila, opsins were recently implicated in larval temperature preference behaviour and in audition of adult flies. Drosophila larvae also require visual opsins for locomotion. The proprioceptors that control locomotion express these opsins. Opsin-deficient mutant larvae have reduced muscle contraction amplitude, locomotion speed, and crawling agility. Opsin-dependent locomotion defects closely resemble the locomotion deficits of mutants whose chordotonal neurons are impaired. Promoter-fusion experiments and electron micrographs revealed that the sub-cellular localisation of mechanosensory channels and the ultrastructure of chordotonal organs is disrupted in the absence of opsins. Furthermore, we can show that NompC, a formerly mechanosensory associated protein, is expressed in three of the six thermosensory cells of the arista. Via heterozygous expression experiments, we determined that these neurons encode warm temperatures. The genetical modification of NompC abolishes the temperature preference, which is attributed to reduced neuronal temperature responses. Further analysis of the response properties of the aforementioned neurons revealed fast adaptation mechanisms that tune the neurons to respond to temperature changes, rather than to absolute temperatures, further corroborating the hypothesis that these neurons are external temperature sensors.

[P158] Pain receptor adaptability in an evolutionary arms race

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Pain sensation allows an organism to perceive danger, respond appropriately, and avoid additional harm. Some prey organisms capitalize on the pain pathway of their predators by using painful venoms or sprays. Predators, in turn, may evolve resistance to those toxins. These adaptations represent an evolutionary paradox. Selection for higher pain thresholds is likely constrained by the necessity of feeling pain. Predatory grasshopper mice (Onychomys torridus) and pinacate beetles (Eleodes longicollis) are an ideal system for examining an evolutionary arms race showcasing counter-selection against prey defenses through modifications to the pain pathway. Pinacate beetles evolved a benzoquinone spray that is intolerable for most mammalian predators, but ineffective in deterring grasshopper mice. We recorded predator-prey interactions in a behavioral arena, in which a beetle was paired with one of three closely related species of rodents: a grasshopper mouse (an obligate carnivore), a deer mouse (a facultative insectivore), or a pocket mouse (a granivore). We found that grasshopper mice are much more successful and effective predators compared to deer mice, whereas pocket mice showed no interest in the beetles. Using a two-bottle choice test, we show that grasshopper mice are more willing to drink greater quantities and higher concentrations of benzoquinone than house mice. Taken together, these results suggest genetic and/or physiological variability in the pain receptors targeted by benzoquinone. We identify TRPA1 as a potential adaptive site, as it is a conserved pain receptor critical for detecting benzoquinone and other noxious stimuli.

[P159] Schooling behavior of giant danios altered after lateral line system ablation

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Schooling fish use multiple sensory systems to maintain position and speed within a school, and disabling their flow sensing lateral line system is known to alter schooling behavior. Previous studies investigating the lateral line's role in schooling only ablated the posterior portion, leaving the anterior lateral line system intact. Revisiting these experiments using partial or complete ablations of the lateral line system would enhance our understanding of its role in schooling behaviors. Here, we examined schooling behavior in fish immediately after their anterior, posterior, or complete lateral line systems were ablated with aminoglycoside antibiotics, and at weekly intervals after the system regenerated. We filmed schools of five giant danios, Devario aequipinnatus, with two high-speed cameras and reconstructed the 3D positions of each fish within a school. Fluorescent staining (4-Di-2-ASP) of hair cells that comprise the lateral line system confirmed the ablation from the antibiotic treatment, and regeneration of the hair cells after one week. As the treated fish swam within the school, we found that fish with their anterior and entire lateral line system ablated were able to maintain a normal position within the school immediately after the lateral line ablation, but these fish could not school normally one and two weeks after treatment even though the hair cells were functional as indicated by fluorescent staining. By four weeks post-treatment, the treated fish could again school normally. These results suggest that fish may need more time behaviorally to adjust to processing signals from newly regenerated hair cells of the lateral line system.

[P161] Retinal horizontal cells express Cry4: a new take on the avian light-dependent magnetic compass

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It is well known that birds can detect the Earth's magnetic field and that they use a light-dependent, radical-pair-based magnetic compass for orientation. Cryptochromes (Cry) located in the retina of birds have been suggested as the magnetoreceptors, since they are the only known vertebrate photopigments known to form radical-pairs upon photo-activation. Vertebrate-type Cry1 and Cry2, however, are believed to not bind FAD, the photosensitive cofactor of cryptochromes, and may therefore not be involved in light-dependent magnetoreception. Cry4, on the other hand, has retained the ability to bind FAD in vivo, thus is intrinsically photosensitive, which is crucial for the radical-pair mechanism to work. To investigate the location and distribution of Cry4 in the avian eye, we used immunohistochemistry on thin sections of zebra finch retinas. These birds use a light-dependent magnetic compass and express Cry4 in their retinas at constant levels. We found Cry4 expression in a subpopulation of horizontal cells in the retina, known to modulate the input from rods and cones to enhance contrast and aid in colour opponency. The involvement of horizontal cells in light-dependent magnetic compass reception provides intriguing evidence for a direct interaction of magnetic compass information with the visual system. Moreover, the observed distribution of Cry4 in only parts of the retina suggests a mechanism that provides orientation information but does not interfere with important visual tasks, like feeding or predator avoidance.

[P162] Evidence for a dorsoventral visual and tactile sensory complementation for nocturnal foraging in the Band-winged Nightjar (Systellura longirostris)

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Nocturnal birds have many anatomical and behavioral specializations, related to foraging in low light conditions. One of those strategies is the complementation of low intensity visual stimuli with auditory, tactile or olfactory cues. Nightjars are nocturnal-crepuscular foragers, that feed on flying insects by aerial pursuit. Although the neuroanatomical basis for this phenomenon is still unknown, behavioral evidence suggests that a vision-only mechanism might not be sufficient to account for nocturnal foraging. The Band-winged Nightjar (Systellura longirostris) has a conspicuous rictal bristle system, with long ventrally oriented facial feathers. Those structures could have a sensory role, related to prey detection, but no evidence of mechanoreceptors has been described in this species. If that evidence is found, this could be an instance of visual and tactile complementation.We analyzed the neuroanatomy of visual and rictal bristle systems in the Band-winged Nightjar, to determine the presence, characteristics and neural projections of mechanoreceptors, and the possible relation between prey capture events and sensory systems disposition. We found abundant Herbst corpuscles, concentrated in bundles, and laterally distributed around each bristle follicle, with a decreasing nasal-to-temporal density gradient. Using CTB tracing, we found that those corpuscles project to several posterior nuclei, part of the PrV system. Maximum visual binocular convergence and retinal ganglionar cell density occurs at areas corresponding to the dorsal portion of the visual field. These results support a multimodal dorsoventral complementation scenario, coherent with the behavioral foraging sequence; initial visual detection triggered in the dorsal visual field, and a visual-independent capture event, mediated by the ventrally oriented tactile rictal bristles.

[P163] Is Piezo protein the mechanotransduction channel in spider Cupiennius salei mechanosensilla?

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Mechanotransduction is essential for detecting external mechanical signals, such as touch, vibration and sound, as well as for sensing internal forces including stretch in the pulmonary and cardiovascular systems. Mechanical stimuli create electrical signals by opening mechanosensitive ion channels. Recent evidence suggests that evolutionally conserved large pore forming Piezo proteins play crucial roles in mammalian and insect mechanosensory processes, including cell volume regulation and sensory transduction. The tropical wandering spider, Cupiennius salei, provides a model of mechanotransduction due to accessibility of its mechanosensory neurons for experimental manipulation. Our analysis of the spider transcriptome revealed one gene encoding a Piezo protein, CsPiezo. In-situ hybridization with a specific probe found this gene expressed in all mechanosensory neurons in spider legs. A custom-made polyclonal antibody detected a single specific band at 295 kDa, the predicted molecular weight of CsPiezo. We tested this antibody using fluorescence immunocytochemistry in whole-mount preparations of the patellar hypodermis as well as vibratome sections of the spider brain. In the hypodermis, axons and dendrites of all mechanosensory neurons were strongly labeled, with no labeling in epithelial or glial cells. Many large cell bodies in the subesophageal and supraesophageal ganglia were also labeled, as were nerve fibers projecting to the periphery. Since the mechanotransduction channel that detects touch and vibration in spider mechanosensory neurons is believed to be located exclusively in their dendritic tips, it seems unlikely that CsPiezo forms the mechanotransduction channel. It is more likely to have other, more general, functions in sensory and other neurons.

[P164] Single Sensory Neurons Encode Haltere Motion

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The ability of dipteran insects (flies) to perform complex acrobatic maneuvers in flight is due in part to specialized sensory organs called halteres. Halteres are modified hindwings that oscillate in antiphase with the forewings during flight and detect forces produced by body rotations. One of the strongest of these forces is the Coriolis force, which causes torsion at the base of the haltere and can change the haltere's trajectory. How do the sensory neurons of the haltere encode this force information? The activity of haltere primary afferent neurons has been measured previously using motors to oscillate the haltere. However, these experiments only included haltere motion in a single oscillatory plane. During a body rotation, the trajectory of the haltere changes and a large component of that change is a lateral displacement (Thompson et al., 2009). Here, we used sharp intracellular electrodes to record from primary sensory neurons while oscillating the haltere at different angles (lateral displacements) relative to the body, as well as during voluntary haltere oscillations. We found that the activity of individual sensory neurons changes with lateral displacement. Lateral displacement had a significant effect on the phase of activation, as well as on the amplitude and frequency threshold at which a cell will respond. From these results, we propose a possible mechanism by which haltere neurons could encode lateral movements during flight.

Olfaction & Taste

[P165] Functional analysis of an inhibitory microcircuit in the Drosophila mushroom body calyx

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The insect mushroom body (MB) is an important learning and memory center located at the third layer of the olfactory circuit. Olfactory stimuli evoke sparse, decorrelated response patterns within the population of Kenyon cells (KCs) that compose the MB. We are investigating the structural and functional connectivity that enables KCs to take broadly tuned synaptic inputs and convert them into narrowly tuned output. Olfactory input to the MB comes from projection neurons (PNs) in the glomerular layer of the system. PN boutons form synapses with KCs called microglomerular complexes. These are composed of the PN bouton, surrounded by claws of several KCs, along with other pre- and post-synaptic elements, including GABAergic neurons. EM reconstruction of one of these microglomeruli by the Bock and Tavosanis labs has identified all contributing synaptic partners. One of these is the anterior paired lateral (APL) neuron, a GABAergic neuron which has been previously shown to promote sparse coding in the KC layer (Lin et al. 2014, doi:10.1038/nn.3660). Based on the EM reconstruction, we hypothesize that APL could potentially provide local input and output within a microglomerulus, which could have important consequences for signal processing at this stage of the brain. We examine functional response properties of APL processes within the MB calyx using in vivo two-photon calcium imaging. We examine responses both to sensory stimulation and optogenetic activation of PN inputs. Results of this study will give insight into the function of inhibitory feedback and its contribution of odor coding both at the level of the KC population and at the level of the microglomerulus.

[P166] Neurobiological indicators of olfactory sensitivity in cartilaginous and bony fishes

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The size (volume and mass) of the olfactory bulbs in relation to the whole brain is currently used as a neuroanatomical proxy for olfactory abilities in different vertebrates, including fishes. The olfactory inputs to the brain have not yet been quantified in any species of fish; although, the number of neurons could provide an estimate of the computational power of this sensory modality. Here, we present a comprehensive assessment of anatomical proxies of olfactory sensitivity in two model species, the brownbanded bamboo shark (Chiloscyllium punctatum) and the common goldfish (Carassius auratus), using immunohistochemistry, microscopy and bioimaging techniques. The relative size of the different components of the olfactory pathway was assessed using X-ray-based microcomputed tomography imaging with an iodine-based contrast enhancement (diceCT). Primary and secondary olfactory neurons were quantified using transmission electron microscopy to estimate the level of convergence (ratio) between the input to the olfactory bulb and the input to the telencephalon. Quantitative and volumetric data were standardised to propose a more accurate proxy for olfactory sensitivity.

We found that the bamboo shark has a relatively larger olfactory bulb and a higher convergence ratio of olfactory inputs in the olfactory bulb than the goldfish. Ultimately, quantifying neuronal inputs combined with volumetric analysis would better predict the relative importance of olfactory sensitivity in fishes, which is fundamental to improving our understanding of the main drivers underlying the evolution of the vertebrate brain and associated behavioural outputs.

[P67] Chemosensory pathways in amblypygids (whip spiders)

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Arachnids (spiders, scorpions and their kin) are best known for their sense of touch and vibration. Much less is known about their chemical senses, the exception being chemoreceptors on their legs and other body appendages. In many animal phyla, afferent chemosensory neurons terminate in clusters of globular structures called glomeruli. Like other arachnids, whip spiders have eight legs, but the first pair is modified into extremely long and thin sense organs ('antenniform' legs) covered in large numbers of mechanosensory and chemosensory sensilla that sweep across and probe the environment. Chemosensory neurons terminate in about 700 glomeruli in the neuromere associated with the antenniform leg (analogous to the vertebrate olfactory bulb or insect antennal lobes). From there, olfactory interneurons ascend to the brain proper and innervate a set of about 600 second order glomeruli. The similar numbers of primary and secondary glomeruli suggest an almost 1:1 relationship, potentially indicating that the secondary glomeruli maintain odorant specificity but extract different aspects of information from the sensory input. A third, adjacent layer of smaller and much more numerous glomeruli (ca. 6000) appear to extract further information from the chemosensory input. These secondary and tertiary glomeruli are associated with unusually large and complex mushroom bodies, brain components known in insects to be involved in learning and memory. The extreme complexity of the chemosensory pathway of amlypygids is reminiscent of complex visual processing underlying navigation in vertebrates and insects and supports hypotheses of advanced chemosensory navigation capacities of whip spiders.

[P170] Pheromone utilisation in elasmobranchs

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Pheromones are chemicals cues that pass from an individual to be detected by the chemosensory systems of a conspecific and are known to mediate behavioural and physiological changes. Pheromones responsible for migration, endocrinological synchronisation, aggression and mate attraction and arousal have been well documented for teleosts, however, comparable studies addressing the presence, structure and activity of elasmobranch-derived pheromones is lacking. Observational reports of adults in both captivity and the natural environment suggest elasmobranchs use chemical signals for attracting and pursuing mates although little work has been conducted to characterise the compounds responsible. To better understand elasmobranch communication, behaviour and reproduction a detailed investigation of chemical products and the underlying neural mechanisms involved in signal detection and integration is warranted. In light of these questions, and, using the epaulette shark, Hemiscyllium ocellatum as a model species, preliminary results of an extensive chemical analysis of animal holding water are reported. Additionally, initial immunohistochemical and histological observations of the morphology, density and distribution of the olfactory receptor neurons likely responsible for pheromone detection are presented with a brief discussion of future electrophysiological and behavioural work.

[P171] Multiple sensory organs employ active ciliary-driven suction in nudibranch gastropods

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Nudibranchs use mechanosensory and chemosensory cues to localize food, predators, and mates. Despite studies on odor driven behaviors, little is known about the invaginated and ciliated grooves on the oral veil. Prior work indicates that nudibranchs detect odor and flow cues with their rhinophores and we here investigate the ciliated grooves, a potential boundary-layer sniffing olfactory organ. Many species generated ciliary-driven currents between the branches of the clavus of the rhinophore that allowed the animal to sample media from up to 5mm away from the organ, likely reducing the olfactory impact of the boundary layer. The ciliated grooves are located in front of the animal facing downward towards the substrate such that odors trapped in the boundary layer might be actively pulled out of the layer and into the groove. Inside the grooves at the lateral ends of the oral veil are dense beds of motile cilia similar to those found on the rhinophores and foot. The outer edges outside the groove have patches of cilia, though it is unclear if they are specialized to be touch or taste receptors. Particle image velocimetry showed active particle movement through beating of the cilia. We traced the nerves from the ciliated groove to the cerebral-pleural complex and the pedal ganglia in several species. Nerves from the cerebral and from the ventral, anterior pedal ganglia innervate the oral groove ipsilaterally. Stimulation of rhinophore nerves and single neurons in the pedal ganglia have given insight to how particle flow speed may be controlled by modulating the beat frequency of cilia.

[P172] Experience- and context-dependent modulation of aggression behavior in ants

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Ants rigorously defend their nest against foreign individuals to protect their colony's resources from being exploited. The recognition of nestmates and discrimination from non-nestmates is based on mixtures of low-volatile components of cuticular hydrocarbons (CHCs) which are species- and colony-specific. Trophallaxis and allogrooming result in mutual exchange of CHCs and homogenization of CHC profiles among nestmates. However, the CHC profiles of workers within a colony show variation that relate to different tasks they perform. It is generally assumed that encountering workers compare the perceived CHC profile (label) of the opponent with own neural representation for nestmates (template) and mismatches between label and template allow discrimination of nestmates and non-nestmates. Importantly, labels change over time since they are subject to environmental influence, e.g. nest material and diet. Hence, workers need to constantly update their template. In our studies, we address the questions how social context (interactions with nestmates) modulates individual propensity to react aggressively towards non-nestmates, and how systematic modifications of the CHC profiles (added or removed components) impact the recognition process. Our results demonstrate that the nestmate recognition system is suited to mediate an adaptive and flexible resource defense. The variety and chemical similarity of labels that ants can discriminate strongly indicates that rather than a global template for 'nestmates', workers have several templates available to recognize different types of nestmates. We propose that the nestmate recognition system of ants is partitioned, where nestmate-specific labels are learned as entities and different templates, each having its own specificity range are formed.

[P173] Neural representation of spatial odour perception in the American cockroach

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The survival of most animal species depends on an efficient processing of olfactory information, an aspect of particular importance for insects living in dark and non-turbulent environment such as the American cockroach. Morphological and electrophysiological analysis revealed that pheromone receptor neurons innervate the cockroach macroglomerulus (MG) preserving an antennotopic distribution. Furthermore, MG output neurons have a stereotyped subglomerular distribution, and a specific receptive field on the antenna. These findings provide the first clear indication that the spatial distribution of an odorant can be encoded within the olfactory circuit. Here, by labelling antennal lobe projection neurons with a calcium sensitive dye, we investigate the intensity and subglomerular distribution of the neural activity within the MG, when stimulating different portions of the antenna. In addition, to assess the behavioural relevance for spatial stimulus location, we indirectly measured the motor response to proximal/distal antennal stimulation by electrophysiological recordings of the neural activity in the ventral connectives.

[P174] Fruit flies use stimulus onset asynchrony for odour source separation

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Many animals depend on olfaction to locate resources, mates or predators. However, in a natural environment, the components of relevant odours intermingle with those from other sources. Therefore, in order to track an odour source, animals first need to separate odorants from different sources. To do so, animals could use temporal stimulus cues, as odorants from the same source exhibit correlated fluctuations, while odorants from different sources are less correlated.
Here we test the hypothesis that Drosophila can use these fine-scale temporal differences in odorant fluctuations for odour source separation. We mimicked odorants from one or two sources by presenting binary mixtures of odorants with synchronous onsets (as is the case for one source odours) or asynchronous onsets (two source odours). Using a free-flying behavioural odour-tracking paradigm, we demonstrated that Drosophila were able to separate mixtures of attractive and aversive odorants based on a stimulus onset asynchrony of just 33 ms. These results indicate that the insect olfactory system can resolve the timing of odorant stimuli with high temporal precision and use stimulus timing for odour source separation, similar to the vertebrate auditory or visual systems which use stimulus timing for concurrent sound segregation or figure-ground segregation. Furthermore, the observed rapid olfactory processing poses temporal constraints on the neural code for odours, implying that the insect olfactory system may rely on a fast and precise coding mechanism.

[P175] Mechanisms enabling better discrimination of transients can cause decorrelation in the antennal lobe

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Information is lost as it crosses synapses. ORNs are thought to synapse onto the antennal lobe (AL) rather than directly to the calyx of the mushroom body because of two major features of the AL : (a) the convergence of ORNs expressing same receptor type and thresholding, thus improving SNR (b) transformation of representation mediated by the local interneurons that is thought to have major roles in information processing. Among the hypothesised functions of LN, decorrelation is thought to improve the discrimination performance of the organism as a whole. We show that weight matrices with identical strengths for all synapses of a given type is sufficient to satisfy the experimentally observed behaviour of PN, ORN and their local field potentials (LFP). In a sum and threshold classifier analogous to the KC, PN representations performed as well as the ORNs during the onset and offset of the odor but not during the steady state and this effect was mediated by slow inhibitory currents (SICs), thought to be mediated by GABAb. The same SIC mediate the observed decorrelation, but the classification performance was not correlated with decorrelation at all time points. This is consistent with the view that encoding onsets and offsets while suppressing steady states is the major role of SIC in the AL network and decorrelation is observed as a result of this.

[P176] Male African clawed frogs show olfactory responses to socially-relevant stimuli

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The African clawed frog (Xenopus laevis) is fully aquatic, living in murky ponds in sub-Saharan Africa for its entire life cycle. It has unique nasal anatomy with a principle chamber, or “air nose,” that can detect odorants from breathed air, as well as a separate chamber, or “water nose,” that can detect odorants from water while submerged. We were interested to know if the water nose could detect conspecific odorants that could potentially be used as social cues. To investigate this question, we developed an electro-olfactogram (EOG) recording technique for this species, placing an electrode along the olfactory epithelium in the water nose chamber of adult male frogs, and recording receptor potentials while delivering stimuli. Stimuli included skin secretions and cloacal fluids from male and female conspecifics, as well as amino acids (methionine and alanine) and saline as controls. We tested multiple dilutions of each stimulus, allowing us to estimate the sensitivity of the olfactory response. We found robust responses to conspecific stimuli and amino acids. Responses were evoked reliably for conspecific stimuli diluted 1000 fold or more. On average, EOG showed greater sensitivity to cloacal fluids than skin secretions, but responses to stimuli derived from male and female animals were similar. We conclude that these frogs have the capacity to detect olfactory stimuli from conspecific animals in the water. The identity of the stimulus molecules being detected, and the relevance or utility of this olfactory information for social behavior, are as yet unknown.

[P177] Sensory mechanisms for localizing spermatophores in the axolotl (Ambystoma mexicanum), an aquatic salamander

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Salamanders transfer sperm externally using a gelatinous capsule of sperm called a spermatophore. Sperm transfer between axolotls is initiated when the female starts to follow the male. The male deposits a spermatophore then continues to walk forward with the female close behind. The female then picks up the spermatophore with her cloaca, fertilizing her eggs internally. How does the female determine the location of the spermatophore? We predict that female axolotls rely on two sources of cues: visual, tactile, and chemical cues from spermatophores, and tactile feedback from males. We collected spermatophores from courting axolotls and then introduced a spermatophore into an aquarium containing a different female. Female axolotls exposed to spermatophores exhibited longer, more frequent bouts of a courtship behavior called tail fanning than did control females not exposed to spermatophores. This result suggests that spermatophores provide sensory cues important for courtship in female axolotls. Males are another potential source of cues. A male occasionally provides a female with tactile feedback while she is following him by displacing her with his tail, hindlimbs, or cloaca. This physical contact may serve to correct the female’s trajectory and properly align her cloaca with the spermatophore. We found that in cases where this tactile feedback occurs, females were more likely to acquire spermatophores. These experiments help elucidate the sensory mechanisms that female salamanders use during spermatophore localization. More broadly, this research provides insight into the evolution of external sperm transfer in salamanders and the diversification of mating strategies amongst amphibians

[P178] Disparate wiring principles in the air- and water-smelling regions of an amphibian olfactory system

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Olfaction is essential for animals both in aquatic and terrestrial environments. Amphibians were the first vertebrates to rise to the evolutionary challenge of developing a sense of smell that identifies chemical stimuli in the two different media (water and air). Their transitory position between water and land is mirrored in their bi-phasic lifestyle with aquatic larvae transforming into mostly terrestrial adults. Using neuronal tracing techniques paired with functional calcium imaging, we investigated how the olfactory system changes during the development of Xenopus laevis and how these changes reflect adaptations to detect both aquatic and terrestrial odors. In the aquatic larva, the olfactory epithelium in the principal nasal cavity detects waterborne odors. During metamorphosis it is remodeled and transformed into the adult air nose while a new sensory epithelium develops in the middle cavity - the adult water nose. The adult middle cavity connects to the ventral portion of the olfactory bulb while the reorganized principal cavity projects to an expanding dorsal part, appertaining to the air-smelling system. In contrast to cellular wiring in the water system, receptor neuron axons in the dorsal air bulb often cross between the bulb-hemispheres; Additionally, a single postsynaptic projection neuron is getting input from both nostrils. This alternative wiring principle suggests an additional integrative function of the air-system that might be of behavioral relevance. The development and adaptations of the olfactory system in amphibians provides valuable insights into the evolutionary processes necessary for a successful transition from water to land.

[P179] Neuroethology of chemosensory-based navigation behaviour in the aquatic gastropods Tritonia and Lymnaea

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In aquatic gastropods, various cues guide navigation behaviour with respect to prey, conspecifics and predators. Odours, flow, light, magnetic fields, and others may be integrated to control crawling. Our goal is to study this integrative sensory processing with three different projects. 1) In turbulent odour plumes, the nudibranch Tritonia diomedea uses odour-gated rheotaxis to find odour sources. Somewhat surprisingly, this behaviour requires just one of the paired cephalic sensory organs (rhinophores). We tested whether inputs from two rhinophores would be beneficial for navigation in challenging odour plumes, with reduced odour concentrations or flow disruptions. Our initial results show few differences between animals with one or two rhinophores, suggesting that any benefits of two inputs for navigation were subtle at most. 2) The pond snail Lymnaea stagnalis lives in varying flow environments. We compared navigational performance of the snails between still water and flowing water that spanned both laminar and turbulent regimes. Our results show effective navigation towards food sources regardless of flow speed, suggesting the snails may switch between chemotaxis and odour-gated rheotaxis depending on flow conditions. 3) The peripheral nervous system plays a critical but unexplored role in sensory processing in gastropods. We are establishing expression patterns of neural-specific genes with quantitative PCR and in-situ hybridization of as an alternate approach to study neural structure and function in L. stagnalis. In the longer term, we anticipate these projects converging to help understand how peripheral neural circuits contribute to the control of navigation behaviour in gastropods.

Ecology

[P180] Habitat light intensity and the color of Anolis dewlaps

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Anolis lizards communicate with throat fans (dewlaps) that vary in color among species. Anolis habitats range from desert to dense forest. It has been hypothesized that selection for optimal visibility under different light conditions might, to some extent, explain the differences in dewlap color. However, when examined closely, the different habitats have very similar spectral characteristics. We therefore hypothesized that differences in total habitat light intensity might play a role in selecting for dewlap color differences. We used a photoreceptor noise model, as well as a behavioral visual-attention assay, to determine the relationship between dewlap color and visibility, when viewed against typical backgrounds. Both methods showed that under high light conditions, red or orange dewlaps are most visible. However, in low to moderate light, yellow or white dewlaps are most visible. Yellow or white dewlaps reflect and transmit more total photons. Where photon flux is limited, this increases the signal-noise ratio, and makes their color more visible. In the wild, red or orange dewlaps are more common in low shade habitats, while yellow or white dewlaps are more common in shaded habitats. Total light intensity appears to be a more important selective force on dewlap color than habitat spectral properties.

[P181] Testing the acoustic niche hypothesis in a seasonally changing tropical bird assemblage

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Acoustically signaling species encounter competition and masking interference from other vocalizing species, and as a result may occupy distinct regions of time-frequency space to minimize this interference (the acoustic niche hypothesis). In the Asian tropics, bird species composition dramatically changes with the arrival of winter migrants, and their influence on overall singing activity and acoustic niches remains understudied. I recorded avian singing activity patterns using four synchronized microphones in an urban deciduous scrub habitat in Western India, an understudied and highly threatened habitat harboring high bird diversity. By comparing morning singing activity across the monsoon and winter migrant seasons, my study examines seasonal changes in singing activity of individual species and tests whether concurrently vocalizing birds partition acoustic space to avoid competition. At least 78 vocalizing bird species were recorded across seasons, with several distinct seasonal changes in the composition of the acoustic community. Some resident birds (such as cuckoos, weavers and francolins) are highly vocal in the monsoon season, and silent/absent during migrant season, whereas others (such as shrikes and yellow-eyed babblers) increase their vocal activity during migrant season, possibly due to local movements into the study area. Additionally, a number of vocal migrant birds (particularly warblers and flycatchers) were recorded during the winter season. Preliminary analyses suggest that sympatric closely-related species (eg: Cuculidae, Cisticolidae) occupy distinct regions of time-frequency space. This study thus represents some of the first evidence of avian acoustic niches from India's semiarid scrub habitats.

[P182] Quantifying environmental constraints on the signaling strategies of Australia's dragons

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To appreciate fully the forces that shape the behaviour of animals, it is necessary to understand the information-processing tasks under evolution relevant conditions. Knowledge of the environment in which animals operate and the sensory processing demands that mediate behaviour are crucial. Two fundamental questions arise: How are natural, biologically relevant stimuli distinguished from extraneous noise? What are the sensory limitations that necessitate a change in behaviour? Animal signals are a useful system to understand the sensory and ecological limitations that govern behaviour, but require detailed descriptions of signal structure with careful analysis of the structure and dynamics of environmental noise within which those signals must function. The use of movement to communicate is widespread in the animal kingdom, including our own attempts to attract the attention of others by waving our arms (and our efforts to enhance this signal in crowded places). However, until recently, descriptions of the perceptual and environmental constraints on motion signalling do not adequately address the fundamental questions proposed above. We are using an innovative approach that combines field techniques with tools from 3D animation and computer vision to determine how habitat structure, weather and motion vision influence motion signaling of Australia's charismatic dragon lizards. Using sophisticated 3D animations, we quantified how signal effectiveness is affected by prevailing wind and light conditions, but also the extent to which animals could mitigate these effects with minor adjustments to signal structure.

[P183] Evidence for differential aromatase gene promoter methylation in a cichlid with pH-influenced sex determination

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The sex determination mechanism of most species has evolved to maintain a 50:50 sex ratio, but an even mix of males and females in every brood is not required. The Charnov-Bull model predicts that environmental sex determination should be selected for when early life environmental conditions differentially impact males and females. In the African cichlid fish Pelvicachromis pulcher, sex determination is influenced by water pH during the first 30 days of life, producing a male bias at lower (acidic) pH and a female bias at neutral pH. pH also impacts ratios of the two common male morphs, which differ in color and reproductive behavior. Methylation of the gonadal aromatase gene (cyp19a1A) has been linked with temperature sex determination in other teleost species, but has not previously been investigated as a mechanism for pH dependent sex determination. We report that methylation of the cyp19a1A promoter differs between fish raised in acidic and neutral conditions, consistent with a role in pH sex determination. In addition, we report on tissue-specific methylation exhibited by the cyp19a1A gene promoter as well as the brain aromatase (cyp19a1B) gene promoter.

[P184] How efficient neural processing influences mimicry, mate choice and aesthetics in animals?

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Why many animals are attracted by highly ornamented sexual partners, and how extravagant signaling traits such as aposematic displays evolve has remained poorly understood. Here, we will explain why the efficient processing of neural signal (EPNS) is a fundamental yet overlooked driver of the evolution of communication. We will first present psychological and neurophysiological evidence from the field of neuroaesthetics showing that, in humans, the attractiveness of a stimulus is strongly linked to EPNS. Then, we will show that artificial, hierarchical neural networks can predict this link. Because such networks can accurately model various neurophysiological and psychological properties of visual perception in a wide range of Vertebrates, it is now possible to use artificial neural networks to non-invasively investigate the effect of EPNS on behavior and its evolutionary consequences in non-human animals. We will present results demonstrating that EPNS is a key component of mate choice and mimicry in various organisms. We will conclude by discussing the link between EPNS and aesthetics, and thus the potential role of aesthetics in the evolution of animal communication.

[P185] Visual approach computation in feeding hoverflies

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When an animal encounters a potential threat during a foraging bout, it must choose between staying at the current foraging site or leaving in search of a new site. Staying at a threatened foraging site might result in injury or even death, whereas leaving induces lost feeding time, energy expenditure and possible exposure to predators. This trade-off is also present in hoverflies, alternative pollinators that are occasionally predated upon by other insects and that do not readily share flowers even with harmless insects. Here, we have used the hoverfly Eristalis tenax to investigate how they respond when faced with this common foraging trade-off. Using high speed videography and 3D flight path reconstruction we studied the behaviour of foraging female Eristalis hoverflies when approached by other insects. We found that the female hoverflies left the flowers 94% of the times when approached by another insect, and that the speed at which they left the flower was 3 times higher than when they left the flower apparently spontaneously. The incoming insect did not appear to perform an attack, but was more likely focused on the attractive cues of the flower. We found that the foraging hoverfly might use the retinal speed or the angular increment of the incoming insect to determine the time of take-off, which can be further investigated in future laboratory experiments. Understanding the natural ecology of pollinators, including the trade-offs that they face, is becoming increasingly important, as highlighted by recent reports of an alarming decrease in insect biomass.

Computational Modelling

[P186] Tracking wakefulness transitions in Drosophila

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Most techniques to assess the wakefulness states in Drosophila rely on using movement data and label sleep as being immobile for more than 5 minutes. This leads to a binary classification of awake and asleep. These measures are tedious and error prone and are not based on neural data. Here we developed a classification algorithm based on two channel differential local field potential recorded from the fly brain. We first identify features that are capable of differentiating between different wakefulness states using epochs of 1 minute duration. We used normalised wavelet amplitudes, dimension of activation (Magnin et al. 2010) and performed a multi-class support vector machine classification to arrive at labels of awake and sleep. We were able to achieve classification accuracies of up to 88% in train data set and 87% in test data set with 10 flies undergoing 24-hr recordings. Further we also identified a transitionary state that leads into sleep and another state that leads away from sleep. The framework developed here is potentially powerful to provide accurate wakefulness levels along with their transition further leading to a mechanistic description of the changes occurring in functional networks at different wakefulness levels in Drosophila.

[P187] Modeling visual perception, learning, and memory of ants navigating in naturalistic environments

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Through simulation we characterized how visual cues that ants use are extracted, prioritized and stored during navigation. This model simulates navigation in a procedurally generated environment where the visual cues can be precisely characterized. Our algorithms extracted and stored the visual cues that were available during a single Levy walk foraging event. Following a random foraging event, the success on subsequent foraging bouts using the stored information was examined. When we examined subsequent walks we found the success of the simulated ant in finding the goal location using only a particular cue or a combination of cues depended on two factors – the length of the route and decay rate of information in a memory network. Our data suggests that the optimal strategy is to sample and store around 1000 points along the foraging route, independent of scale, with a network subjected to exponential decay. These parameters resulted in a stored representation that allowed the simulated ant to best find the goal on subsequent foraging bouts. We then produced several novel random foraging walks with the same goal location. The subsequent walks for these foraging events had similar success demonstrating sufficient information was stored and resulted in idiosyncratic foraging routes due to the varied information encountered during the random walk. Additionally, we explored how multiple subsequent walks updated and modified memory to produce more robust walks over time. Lastly, we compared the success of subsequent of the model when foraging in sparse and cluttered environments.

[P188] From shark brains to human cerebellum: an evolutionary perspective on pattern formation in the cerebellar granule cell layer

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In the human cerebellum the granule cell layer has 69 billion neurons; that is 80% of the neurons in the brain. What do they do? Why so many? And how could such a multiplicity of neurons self-organize to produce and maintain their required functionality? Understanding of the 'proto'-cerebellum found in shark brains, and comparable work on weakly-electric fish, provides a neuroethology perspective from which to address these questions.

[P189] Neural models of ant navigation in a realistic 3D world

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Desert ants are extremely efficient at localizing their nest while foraging and are able to use visual memory to find the correct direction even on extended routes. One proposed explanation for this ability is that they store multiple views when traversing a route (e.g. when using path integration to return home), and in subsequent homing compute the familiarity of the current view in comparison with views stored in their memory. A computational model of the mushroom body has previously been developed and tested as the possible neural mechanism underlying this navigation strategy. Although it has been shown capable of one-shot learning of foraging routes using sparse coding, its efficiency was not fully tested using ecologically relevant data. For this purpose, we used data collected in real field experiments to reconstruct real world foraging and route following in a 3D virtual environment. The virtual world allows simulation of real ant visual inputs, including UV-green vision and the full sky polarization pattern, as well as reconstructed ground and vegetation meshes. From the field experiments we can import into this virtual world a learning dataset of real ant foraging routes. Our aim is two-fold: on one side, we wish to validate the 3D world as a testbed for realistic ant vision and navigation simulations; on the other, we test the efficiency of the mushroom body model and evaluate the impact of parameters such as compound eye resolution, tilting/pitching caused by travel over uneven ground and the implementation of different search strategies on the final performance.

[P190] A model of the central complex circuit supports path integration during sideways drift and vector addition for novel shortcuts

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Bees make straight return paths to their hive after circuitous outward routes, which may include substantial sideways drift relative to the orientation of the head. This drift poses a problem for maintaining a home vector by integrating velocity, as the directional component is derived from celestial information which will be in head coordinates. Bees can also return to locations they have previously visited using vector memory, and have been observed to take novel shortcuts from one food location to another after these have been learned independently. We have developed a computational model of this behaviour closely based on neuroanatomy of the central complex. Key features of the model include: a distributed representation of the current head direction of the animal across transverse neurons in the protocerebral bridge; integration of direction and speed by columnar neurons connecting the protocerebral bridge and the noduli; and a pattern of columnar offsets in connectivity from these two sets of neurons to a third set which calculates the difference between the current direction and the home vector and provides output to control steering. Using orthogonal axes for optic flow as the speed input to this system allows the animal to perform accurate path integration despite side-slip. The same circuit can easily be adapted to allow the animal to steer back to a location, if it has stored the home vector state experienced when first at that location. As the underlying path integration continues to operate, substituting another such memory at any time will steer the animal directly towards this new location, producing a novel shortcut.

Neuronal Development

[P191] Sex and caste-specific neuroplasticity in ants

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Changes in brain neural circuitry occur at critical life stages, such as during the transition into behavioural maturity and the accompanying changes in sensory experience. As in many social insects, ants have caste and sex-specific tasks that change with age. Young workers typically perform tasks inside the nest such as nursing and then initiate foraging as they mature, alate males do little more than search for mates during their short lifespan, and alate females mate and establish a new colony. In ant workers, age-related neuroplasticity involves structural changes to the mushroom body (MB), a higher-order sensory processing region, at both the macro and synaptic scales. Light-induced neuroplasticity in the MB calyx collar, a visual input region, also occurs in workers; however, it is unknown how age and light affects neural reorganization in alates. We investigated how functionally distinct brain neuropils in workers, alate males and alate females of the Australian sugar ant Camponotus consobrinus change with age and light exposure, with a focus on the extent of neuroplasticity in the MB. Lab reared ants were exposed to 2 days of total darkness, 11 days of total darkness, or 2 days of total darkness along with 2 hours natural light exposure each day. Here, I will show the extent of neural reorganisation in specific brain regions that occurs in reproductive and workers castes of ants with varying age and light exposure duration.

[P192] Using marsupials to investigate calcium activity in the developing cortex in vivo

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In the embryonic and early postnatal brain of rodents, neuronal activity plays key roles in the formation and refinement of cortical circuits. This activity is characterised by transient patterns of depolarisation that engage spatially distinct regions in age- and area- dependent fashions. However, investigations of early cortical activity have been hindered by a lack of in vivo experimental paradigms to measure such activity during prenatal rodent development. To overcome this, we performed 2-photon microscopy in developing joeys of the Australian marsupial fat-tailed dunnart (Sminthopsis crassicaudata). We overexpressed the genetically encoded calcium indicator, GCaMP6s, via in-pouch electoporation and imaged the neocortex of dunnart joeys in vivo, at developmental stages equivalent to intra-uterine rodents and humans. Here, we describe at least three distinct classes of large-scale neuronal activity in the early developing dunnart neocortex, including: a) asynchronous bursts, b) synchronous bursts, and c) travelling waves. Importantly, analogous patterns have been described in early postnatal rodents, indicating that they may represent an evolutionarily conserved phenomenon of mammalian neocortical development. To our knowledge, this presents the first in vivo characterisation of patterns of neuronal activity in the mammalian neocortex at such early developmental stages. We anticipate this paradigm will open the way for future investigations into the neurophysiology of early cortical activity, the role of such activity in the formation of functional circuits, and the generation of in vivo assays to improve our understanding of the relationship between genes and activity in early neocortical development.

[P193] Comparative transcriptomics of neocortical development in marsupials and eutherians

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The six-layered neocortex is exclusively present in mammals and mediates sensory-motor and higher-order functions. Whereas all mammals share features of neocortical development and connectivity, eutherian mammals differ from monotremes and marsupials in having evolved the corpus callosum. Moreover, most stages of brain development occur during extended placentation in eutherians, whereas in marsupials most brain development occurs postnatally after early development of motor and sensory brain areas required for finding the teat. Very little is understood, however, about the molecular changes that have contributed to the evolution of mammalian cortical development and wiring. To study this, we established a high-depth de novo transcriptomic database of the whole neocortex in mouse and the marsupial fat-tailed dunnart (Sminthopsis crassicaudata), at two equivalent stages of early- and mid-cortical development (E12.5 and E16, and P12 and P20, respectively). Interspecies gene ontology analyses at the younger age showed higher expression of genes in dunnarts associated with epigenetic control and early-onset neuronal development, including neuronal projection formation and synapse-related functions. In contrast, the later age showed higher expression of genes in mice associated with more advanced neuronal cell growth and differentiation, including mitochondrial development, likely a result of the more protracted rate of development of the marsupial brain relative to eutherians. Other genes differentially expressed between species and developmental stages included transcription factors, RNA regulatory elements, axon guidance genes and intracellular signalling pathways. These findings highlight changes in molecular expression that may underlie the evolutionary origin of the corpus callosum.

[P194] Body pattern changes and neural connectivity of a reef cuttlefish across the lifespan

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Cuttlefish are renowned for their remarkable dynamic colouration, colour-blind mimicry, and signal communication. Nonetheless, the neural mechanisms behind these adaptive colourations remain uncertain. Through field and behavioural observations in the cuttlefish, Sepia plangon, we identified 16 body colouration patterns in adults, 10 in juvenile and five in hatchlings. Furthermore, using diffusion magnetic resonance imagery (dMRI) revealed noteworthy details about the gross neural anatomy, and the underlying neural network within the cuttlefish brain. High-resolution MRI (16.4 T) data uncovered a detailed and complete view of the cuttlefish brain (isotropic resolution 30 µm). This also provided accurate estimates of volumetric growth throughout the different life stages, and the identification of 47 lobes with their associated neural connectivity. After further examination of the colouration control centres, the chromatophore lobes (Ch), we found three structural connection pathways. Two of these were derived from the anterior chromatophore lobes (aCh) and were found to control colouration on the head and arms. The other pathway originated in the posterior chromatophore lobes (pCh) and was responsible for colouration of the body. The relative size of the aCh in relation to the entire brain volume was consistent throughout the life stages, at approximately 0.5%. However, the pCh increased to nearly 2% at the maturity stage, suggesting the enlargement of the pCh could be related to the complexity of courtship displays.

Vision

[P200] The Evolution of Eye Loss in parasitic bat flies (Streblidae and Nycteribiidae)

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Bat flies are obligate, blood-feeding, viviparous parasites of bats (Diptera, Hippoboscoidea, Streblidae and Nycteribiidae). All species have reduced or lost eyes, and there are variations among species in the degree of reduction from a maximum of ~50 facets to complete eye loss. In order to study the evolution of eye reduction we have characterized the eye fields of 31 species representing ~65% of the described genera using SEM imaging. To assess the evolutionary trajectory of eye loss, eye field traits (i.e. number of facets, eye field size, facet diameter, facet shape) have been mapped onto a well-supported phylogeny. Ancestral state reconstructions suggest that reduction and eye loss has occurred independently in at least 5 lineages within the two families, with the appearance of novel eye morphologies in some lineages as the number of facets are reduced. In addition, to assess the functionality of extremely reduced eyes, we also measured photoreceptor absorbance in a single species, T. frequens, using microspectrophotometry (MSP). MSP confirmed that the highly reduced photoreceptors are light sensitive with a peak absorbance at 463 nm. Based on preliminary RNAseq data from an additional six species (1 Nycteribiidae, 5 Streblidae), this absorbance is most likely due to expression of an Rh1 opsin. Continuing studies are aimed at assessing the variation in opsin expression patterns and the underlying photoreceptor arrangement.

[P201] Opsin genes: adaptations to different light environments in West African crater lake cichlids

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Fish are known to adapt their visual sensory systems to the different conditions of the aquatic environment. Cichlid fishes provide an excellent model to study such visual adaptations as they colonized the clear and deep lakes and have evolved into multiple endemic species. In this study, we examined and compared the molecular basis of visual systems in two monophyletic cichlid flocks from West Africa: Barombi Mbo (11 species) and Bermin (12 species) crater lakes in Cameroon, and we focused on evolution of the cone opsin genes, i.e. SWS1, SWS2A, SWS2B, RH2Aα, RH2Aβ, RH2B and LWS. We found that colour vision in Barombi Mbo cichlids inhabiting shallow water is based on the expression of mostly three opsin genes: blue-sensitive SWS2A, green-sensitive RH2Aβ and red-sensitive LWS, while in two deep-water species: Konia dikume and Myaka myaka, the red-sensitive LWS opsin is completely missing and instead, colour vision is based on the two green-sensitive RH2Aα and RH2Aβ opsins, and the blue-sensitive SWS2A. We therefore present the evidence for replacement of one gene (LWS) by another (RH2Aα), an adaptation caused by different light conditions. Interestingly, in the Bermin lake cichlids, the proportion of the green-sensitive opsins is much lower than in the Barombi Mbo cichlids. Furthemore, we found that two ecological and colour morphs of Coptodon flava differ in the UV-sensitive opsin gene expression. Our results indicate that adaptation of the visual sensitivity to the different light environments happened mainly on the gene expression level, while the DNA sequence among species remain very similar.

[P202] Now you see me, now you don't: Evolution of the visual sensory system in deep-sea fishes from the Sargasso Sea

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Deep sea fishes have evolved unique physiological and morphological adaptations of vision to their extreme environment. Only recent efforts, however, also revealed molecular adaptations (e.g. loss of the red and UV sensitive opsin genes in many species, amino-acid tuning leading to shifted sensitivity of the opsin protein, or genomes containing up to 40 visual opsin genes). We performed the RNA sequencing to obtain retinal transcriptomes for a diversity of deep sea fishes from the Sargasso Sea and we report the palette of opsin genes in their retina. We further compared the visual system of several species in both larval and adult developmental stages, because while adults live in the deep, their larvae are known to live in shallow pelagic zone, and are thus subjected to very different light conditions, and selective pressures. Namely, we present the differences in the larvae and adults of Idiacanthus fasciola (Stomiiformes), Anoplogaster cornuta (Trachichthyiformes) and Scopelarchus sp. (Aulopiformes). The presented findings on the molecular evolution and ontogenetic plasticity of vision provide significant insights into our understanding of evolution of the sensory systems in an extreme environment. Furthermore, this study deepens our understanding of these highly diverse and charismatic marine vertebrates and throws further light on living in the Earth's "final frontier" – the deep-sea.

[P203] FISHing for Opsins: Photoreceptor Distribution in the Retina of the Cameroonian Crater Lake Cichlid Fishes

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Fish visual system is known for its ability to adapt to the environment. To understand the performance and evolution of visual perception, we focused on the level of spatial photoreceptor distribution in retina. We applied fluorescent in-situ hybridisation (FISH) to investigate retinal organization of cichlids from the crater lake Barombi Mbo (Cameroon) inhabiting different environments, namely deep-water Konia dikume, seasonally deep-water Myaka myaka, and shallow-water species Konia eisentrauti and Stomatepia mariae. The shallow water cichlids express mainly three types of opsin genes: LWS (red wavelengths), RH2A (green; two copies, RH2Aα, RH2Aβ) and SWS2A (blue). Contrarily, both deep-water species lack expression of the red photoreceptor (LWS) in their retinas. Instead, the expression of RH2Aα (i.e. the copy with longer-wavelength maximum) is increased, suggesting the functional replacement of the LWS by RH2Aα. The common cichlid pattern of retinal mosaic consists of single cones, surrounded by four double cones. SWS2A is expressed in the single cones in all species. LWS and RH2A are present in double cones, each in one cell of the double cone. Interestingly, some double cones of K. eisentrauti are also composed purely of the LWS gene expressed in both parts. In the deep-water species, the green-sensitive gene replaced the missing red photoreceptor, as both RH2A genes are found in both parts of double cones in the retina of M. myaka. Photoreceptor distribution of K. dikume remains unclear, but we observed that the size of the photoreceptor cells is generally larger in this species than in the shallow-water species.

[P204] Molecular evolution of vision in the elephant fishes (Mormyridae)

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The elephant fishes (Mormyridae) belonging to the order Osteoglossiformes are mainly known for their ability to use electroreception as an additional sensory system, processing of which probably occupies large portion of the brain capacity. Based on the trade-off principle, vision has therefore traditionally been presented as a sensory system with a minor role in these fishes. However, only scarce research was conducted on the visual system of these fishes, especially on the genomic level. To reveal the molecular basis of vision in the elephant fishes, 10 species from the Sanaga River in Cameroon were investigated. We used the Illumina next-generation sequencing of the whole genomes, as well as the retina transcriptomes to investigate the presence of the different photoreceptor genes (opsins), to quantify their gene expression, and to identify the spectral sensitivities of the encoded opsin proteins (photosensitive to different wavelength based on the DNA sequence). We compared the observed pattern among different species. Furthermore, we focused on species with variable dimension of the eye, namely small eye diameter (mostly genus Mormyrops) as well as relatively large eyes (genus Mormyrus). The observed discrepancies are further hypothesized in the context of different ecological niches, although the biology of most of the studied species remains to be further investigated. These findings

uncover intrinsic adaptations of vision in mormyrids inhabiting mostly turbid freshwaters with limited light conditions. Decrypting vision in the Mormyridae provides therefore new insights in molecular evolution of sensory systems and contribute to the understanding of the biology of these fascinating weakly electric fishes of Africa.

[P205] Visual system and developmental plasticity of European cyprinid fishes from subfamily Leuciscinae

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Adaptation of vision during evolution can heavily impact the biology, reproductive strategy, or feeding ecology of the fish. Teleost fishes posses set of opsin genes encoding for various colour-sensitive proteins in their genome, and always use a subset of those genes in the photoreceptor cells (rods and cones) in retina. Each of these opsins is responsive to a specific wavelength (=different colour in the light spectrum). In this study, we focused on 10 species of cyprinid fishes from subfamily Leuciscinae living in Central Europe. We sequenced the retinal transcriptome and identified the opsin genes participating in colour perception. The most abundant opsin gene in retina is the long-wave sensitive (LWS) gene, encoding for the red-sensitive protein. Such observed pattern may be a result of adaptation to higher level of water turbidity in European rivers with the dominant red light spectrum. Interestingly, several cyprinid species express more than three opsin genes (up to seven) in their retina suggesting probably some co-expression pattern. We compared the amino acid substitutions in the opsin genes in order to identify functional changes in the protein. We have specifically investigated mutations in the key “tuning” sites known to shift the photoreceptor sensitivity, which we identified in all tested opsin genes. We have further focused on the differences in the opsin gene expression between juveniles and adults of the same species in order to identify the plasticity of visual system during ontogeny and we report the comparison of adults and juveniles on five cyprinid species.

[P206] Rapid Evolution of Vision in Sea Snakes

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Sea snakes are among the most ecologically specialised of all squamate reptiles. This group diverged from terrestrial Australian elapid snakes approximately 6-16 MY and radiated into at least 60 species found from open ocean waters to coastal and mangrove habitats. Sea snakes are highly adapted to their marine lifestyle, having paddle-shaped tails, sealed nostrils, and respiratory traits that allow them to remain active underwater for several hours. However, how their visual system adapted to this unique environment is still poorly known. Previous studies suggested a shift from UV sensitive vision to blue sensitivity and photoreceptor transmutation (cones expressing rod-related genes) in two sea snake species. Our results from sequencing visual pigment genes for 151 sea snakes of 49 species suggest that the visual system of sea snakes is highly dynamic. At least 7 transitions are inferred between UV sensitivity and blue sensitivity and these may be linked to diel activity patterns. More interestingly, some species display short-wavelength (UV-Blue) trichromacy, a visual genotype that is rarely reported in vertebrates and may represent a trans-species polymorphism maintained by balancing selection. The fast diversification of the sea snake visual system and novel short-wavelength trichromacy makes this snake lineage exceptional to study the evolution of vision among vertebrates.

[P207] FoxP in bees – from molecules to circuits. Indication for a role in visual processing.

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Three out of four members of the FoxP transcription factor gene family are involved in cognitive functions including language acquisition and dysfunction such as autism spectrum disorder. In zebrafinches and mice FoxP1, FoxP2 and FoxP4 are expressed in comparable brain regions and consequently they have been used as markers for specific neuron types in distantly related vertebrate species. A single FoxP gene locus is present in all metazoan species investigated so far. The DNA-binding sequence of the name-giving forkhead box is highly conserved. Studies in fruit flies revealed that the invertebrate FoxP gene is also necessary for cognitive functions, e.g. decision-making and motor learning. To further analyze the neuronal correlates of these behaviors and to compare neuron types within insects as well as between insects and vertebrates in the frame of deep homology, exploiting FoxP as a marker, we investigated and compared its expression pattern in three bee species (B. terrestris, A. florea and A. mellifera) with a custom-made antiserum. We identified eleven FoxP expressing neuron populations and their neurotransmitters, for A. mellifera also during development. Moreover, we cloned FoxP of A. mellifera and described a splicing alternative previously identified in humans and mice that affects the forkhead box. The largest FoxP expressing cluster (‘mvLO’) does not express this second isoform, whereas the other clusters do. The mvLO connects a visual neuropil (the lobula) with the posteriorlateral protocerebrum and

might be important for visual processing or/and mechanosensory-visual integration. Together, these data suggest parallels and differences for the function of FoxP in bilateria.

[P208] Ommatidial type-specific intra- and inter-cartridge connections in the Papilio lamina revealed by serial block face-scanning electron microscopy (SBF-SEM)

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Insect compound eyes are composed of hexagonally-arranged ommatidia, each containing several photoreceptors. In Papilio, there are three spectrally-heterogeneous types of ommatidia (type I, II and III) that are randomly distributed across the eye. Photoreceptors of each ommatidium project axons to the lamina (the first optic neuropil), where they form a cartridge together with interneurons called LMCs (lamina monopolar cells). The arrangement of cartridges in the lamina preserves the spatial organization of ommatidia in the retina, although the cartridges are rhomboid and thus form a quadrilateral grid. We studied neuronal circuits in the lamina using SBF-SEM. We analyzed all photoreceptors and LMCs in six neighboring cartridges, which correspond to three type I, two type II and one type III ommatidia. While we found numerous ommatidial type-specific intra-cartridge synaptic connections, we also observed quite a lot of synapses between cells of different cartridges, which are absent in Drosophila. The connectivity between cartridges depends on both their ommatidial types and relative positions. Numerous connections exist between cartridges which originate from different ommatidial types and are either directly adjacent or end-to-end neighbors along the dorso-ventral axis (i.e. the six immediate hexagonal neighbors in the retina). Neighboring cartridges of the same type have only around a third as many connections. If two cartridges of different types are end-to-end neighbors along the antero-posterior axis, the number of connections is very low, despite the cartridges' physical proximity. These results indicate that inter-cartridge connections are “heterophilic”, i.e. occur most frequently between neighbors of different spectral types.

[P209] Examination of the histamine hypothesis for a mechanism underlying photoreceptor spectral opponency in the Papilio butterfly

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Spectrally opponent responses, i.e. wavelength-dependent response-polarity inversions, have been observed at the level of photoreceptors quite frequently in butterflies. The opponent photoreceptors show fast-onset hyperpolarization when stimulated with specific wavelengths. Similar fast, on-transient hyperpolarizing responses that are found in the insect second-order visual neurons (lamina monopolar cells, LMCs), are mediated via histamine-gated chloride channels. In the lamina of Papilio, the photoreceptors are not only presynaptic to LMCs, but also pre- and post-synaptic to other photoreceptors. Thus, we hypothesize that histaminergic sign-inverting synapses exist between different spectral receptors as a mechanism for the spectral opponency in butterfly photoreceptors. We conducted immunoelectron microscopic localization on two candidates of histamine-gated chloride channels, PxHClA and PxHClB, in the visual system of the butterfly Papilio xuthus. The anti-PxHClA labeling was associated with the plasma membrane of non-photoreceptor neurons that are postsynaptic to photoreceptors, suggesting that PxHClA is located in the LMCs. The anti-PxHClB labeling overlapped with photoreceptor axons, indicating the PxHClB is expressed at the inter-photoreceptor synapses. Using sharp microelectrode recording in discontinuous single-electrode current-clamp mode, we have shown that the opponent photoreceptor response could be reversed when the membrane potential was close to the equilibrium potential of chloride. We conclude that color vision in Papilio is sharpened up by the inter-photoreceptor opponent signal processing, mediated by the histaminergic chloride channel PxHClB.

[P210] The retinal organisation of a migratory butterfly, Parantica sita

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The chestnut tiger butterfly, Parantica sita, seasonally migrates over thousands of kilometres. Amongst migrating insects, visual cues from the sun including the spectral gradient, brightness gradient and sky polarisation patterns are commonly integrated to form a sun compass allowing accurate orientation during migration. Here, we investigated the primary inputs that may enable navigation by measuring the spectral and polarisation sensitivities of the photoreceptors of P. sita using intracellular recordings. Photoreceptors were sensitive to UV (λmax = 360 nm), blue (λmax = 440 nm) and green light (λmax = 500 nm : 560 nm). The UV and blue receptors were maximally sensitive to vertically polarised light while green receptors were sensitive to diagonally or horizontally polarised light. Polarisation response ratio ranged from 1.2 to 5.2 across the eye with no correlation to either vertical position or spectral sensitivity. Dorso-ventral differences in the tapetal reflection, screening pigments and overall spectral sensitivity of the compound eye suggest that the spectral sensitivity of photoreceptors may differ between regions. Photoreceptors in the ommatidia close to the dorsal rim of the eye possess wider, rectangular rhabdoms, which would provide larger acceptance angle and high polarisation sensitivity possibly optimal for detecting sky polarisation patterns. Additional electrophysiological recordings in photoreceptors and higher-order neurons will enable us to determine which set of visual cues P. sita is using during migration and how this is supported by input from various photoreceptor types.

[P211] A novel method for estimating spatial resolution in compound eyes

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Visual systems play a vital role in guiding the behaviour of animals such as mating, navigating, feeding, and avoiding predators. Estimating the amount and quality of visual information received by an animal is thus key to understanding their behavioural choices. Compound eyes, the dominant eye type in arthropods, are naturally low resolution structures but have the great advantage that they allow flexible control over the shape of the visual field. The ability of compound eyes to resolve spatial detail depends on two factors; the fineness of the mosaic of the receptive elements that sample the image (the inter-ommatidial angle), and the spatial cut-off frequency of the optics. Variation of inter-ommatidial angles across different eye regions as well as different species have been widely used to study sensory adaptations and predict animal behaviour. However, the available techniques for estimating inter-ommatidial angles are difficult, time consuming, and generally require in vivo measurements. Here, we present a new method for estimating inter-ommatidial angles based on Micro-CT images. Using custom-made software programmed in MATLAB we reconstruct in 3D the optical axes of individual ommatidia (crystalline cones) and project these axes into three dimensional space around the animal. The combined fields of view of all ommatidia, estimated from geometrical optics, map inter-ommatidial angles and thus the spatial resolution of the animal across its entire visual field. A main advantage of this method is that it can be applied to preserved specimens allowing analysis of animals which live in unique, but inaccessible environments such as deep-sea crustaceans.

[P212] Spectral sensitivity of the fiddler crab Uca dampieri

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The details of colour vision in fiddler crabs have been debated for almost half a century. Behaviourally, they distinguish between colours but experiments have so far failed to fully explain the physiological basis of this sense. This study measured the spectral sensitivity of Uca dampieri to determine the number of spectrally distinct photoreceptors contributing to the species' colour vision by using flicker electroretinography (ERG) and intracellular electrophysiological methods. The results show the overall spectral sensitivity of U. dampieri peaks at about 440nm and at least two different spectral photoreceptor types are contributing. Experiments uncovered UV sensitive photoreceptors peaking at 350-360nm and at least one type of short-wavelength sensitive photoreceptor (peak sensitivity ca. 440nm). The short-wavelength sensitive photoreceptor(s) appear to be highly variable and their absorption spectra do not fit a standard rhodopsin absorption template. It is not entirely clear whether the short-wavelength sensitive mechanism reflects a single cell type or is composed of multiple cell types, but adaptation experiments cannot separate two spectral classes. However, the study provides clear evidence for diurnal changes in the sensitivity of this short-wavelength mechanism, suggesting the involvement of screening pigments. The most parsimonious explanation of the fiddler crab's retina is that it comprises two types of photoreceptors with peak sensitivities in the 'UV' and 'blue-green' spectral regions, but that screening pigment migration alters the spectral sensitivity of visual photoreceptors. This interpretation explains both the variability of individual physiological recordings and observed diurnal variation in fiddler crab spectral sensitivities, as well as differences between previous studies.

[P213] Crustacean vision: adaptable eyes for extreme changes in light

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Compound eye designs of arthropods are highly specialised to suit the lifestyle and habitat of that animal and there is great variation in eye morphology, internal structure, photopigment, acuity and sensitivity between taxa. Fiddler crabs, Uca tangeri, are subject to extreme changes in ambient light conditions in their tropical mudflat habitats. They must adjust their eye sensitivity to maximise the available light when foraging at night, whilst also protecting them from photo-bleaching in the intense bright conditions of daytime. The mechanisms in which fiddler crabs achieve this and how rapidly or regionally the eyes adjust to extreme changes in light is currently unknown.
In this study, transmission electron microscopy (TEM) and synchrotron X-ray micro-tomography allowed anatomical changes involved with mechanisms for visual adaptation to be observed in the fiddler crab eye. In darkness and especially at night, crabs were found to grow the cross-sectional area of the photosensitive rhabdom, becoming much wider in comparison to the narrow rhabdom of the light-adapted state.
Behavioural experiments showed that the sensitivity of the visual system was enhanced in the dark-adapted eye and there appeared to be a strong circadian influence on the process, in accordance with our anatomical findings. Rapid responses such as 'pupil' changes via screening pigment migrations within the eyes have not

been observed in this species. Instead, results suggest that the changes in adaptation state in response to light-level alterations occur over a relatively slow time course (minutes to hours).

[P214] Polarization sensitivity of ocelli in Australian bull ants

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Acquiring visual information is crucial to make accurate decisions while navigating. In addition to compound eyes, most flying insects possess two or three ocelli, which are small single lens eyes situated on top of their heads. Recent anatomical and physiological evidence in honeybees suggest that ocellar retinae can detect changes in the pattern of polarized skylight to assist in flight control and navigation (Ogawa et al., 2017). Workers of the Australian genus Myrmecia are unusual among ants in possessing three ocelli. Anatomical work has shown multi-directed orientation of microvilli within single photoreceptors, suggesting low polarization sensitivity in ocelli of nocturnal Myrmecia (Narendra and Ribi, 2017). Here, we investigated the spectral and polarization sensitivity of ocelli in nocturnal M. vindex intracellularly using monochromatic lights (340-600 nm). We found two photoreceptor types with peak sensitivities at 360 nm and 500 nm, respectively. Seven out of 15 photoreceptors were polarization sensitive. We then tested behaviourally whether inbound nocturnal M. midas foragers respond to changes in the overhead polarization pattern by rotating a polarizer by ±45°. Half of all intact and ants with occluded ocelli responded to rotational changes in the polarization pattern. Importantly, in the absence of compound eye information, tested by either occluding the entire compound eye or selectively the Dorsal Rim Area, 86% and 67% of ants still responded to e-vector rotations. This indicates that ocelli provide directional information for navigation, which dominates in the absence of landmark information from compound eyes.

[P217] Visual processing in the most complex visual system: In vivo intracellular recordings of interneurons in stomatopod

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Stomatopod crustaceans also known as mantis shrimps has one of the most complex visual system among invertebrates. The compound eye of mantis shrimp consist of two hemispheres and 6 rows of specialized ommatidia termed as midband. The hemispheres are capable of perceiving linear polarized light, Rows 1 through 4 are dedicated for color recognition while rows 5 and 6 are for circularly and elliptical polarized light detection. With such complicated information to encode, it is proposed that stomatopods may perform a distinct visual processing methods. However, the strategies and mechanisms of their visual coding in optic ganglions has only been speculated at morphological and behavioral level. By performing in vivo intracellular recordings with sharp-glass electrodes from optic lobes of Haptosquilla pulchella, we have documented varies interneurons including both spiking and non-spiking laminar monopolar cells (LMCs), tangential cells, dimming cells. Additionally we have unveiled a new category of light-integrating cells that has never been found in other crustaceans. As the first optic neuropil, our investigation has focused on interneurons in laminar layer. At this level, similar to other crustaceans (e.g. crayfish), the LMCs still maintain polarization information while the integrated cells such as tangential cells did not. Additionally, based on different morphologies of responses from LMCs, it is suspected that the lamina ganglionaris are presynaptically inhibited by other interneurons which could be amacrine cells. Though more experiments are required to decode the full visual processing mechanism among different part of eyes, we have achieved a preliminary connection scheme for lamina and medullary interneurons.

[P218] Characteristics of visual interneurons of a mantis shrimp Haptosquilla pulchella

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The compound eye of mantis shrimps consists of at least 16 different classes of photoreceptors. Among them, 14 of which are located in ommatidia organized in a specialized horizontal band of their eyes called mid-band. Due to such a specialized mid-band area, mantis shrimps execute a unique type of eye movement called scanning eye movement. While the anatomy of their visual ganglia closely resembles those of other crustaceans, the neuronal projection of their photoreceptors is distinct to that of all the other crustaceans. To understand how these neuropiles propagate visual information, we use sharp glass microelectrode to record intracellular responses from these visual processing neurons. At least three types of non-spiking neurons are identified. In addition to tonic or phasic neurons that are commonly found in many other arthropods, we have also observed visual responses that resemble temporal integration of the stimulus. These integration neurons gradually yet continuously to depolarize or hyperpolarize for the duration of the stimulus. The membrane potential starts to return to pre-stimulus level only if we turn off the stimulus. To our knowledge, this is the first time such neuron response has been described. Based on the location and neuronal connections of these cells, their functions and relationships to mid-band photoreceptors are speculated.

[P219] Using gene-editing and behaviour to unravel the function of cone cells in reef fish vision

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In the retinae of most vertebrates are both single and double cone cells. Evidence from studies on avian vision suggest that colour vision is mediated by multiple single cones, while luminance vision is mediated by double cones. This division of visual input is unlikely the case in bony (teleost) reef fish that only possess one or two types of single cone and would imply that most are mono- or di-chromats. Moreover, in one species of reef fish the double cones have been shown to contribute towards colour vision. It remains unclear in most species of reef fish which cone types mediate luminance vision, and how single cones contribute towards colour vision. This study will combine the latest gene-editing tools and behavioural techniques to examine how induced changes in cone cell spectral sensitivities cause shifts in the colour vision of the anemonefish, Amphiprion ocellaris. Using CRISPR-Cas9 we will create multiple short-wavelength sensitive (SWS1) and medium-wavelength sensitive (RH2B) opsin knockout-lines. By comparing the visual performance between wild-type (control) fish and transgenic fish in visual detection tasks, it will be possible to isolate the role of single and double cones in anemonefish vision. These findings will improve our understanding on how non-mammalian vertebrate systems have evolved to receive chromatic and achromatic cues. Finally, the multi-technical approach of this study will also provide the first direct examination on how gene-regulatory networks control visual pigment expression in a reef fish. This research is funded by an Australian Research Council Discovery Project Grant (DP180102363).

[P220] Polarization vision for underwater navigation

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The refraction of light through the water's surface and its subsequent scattering generates well-structured patterns in the underwater light field. In particular, the polarization angle of the scattered light outside of Snell's window is highly dependent on the sun's elevation in the sky and the relative heading of the observer. These patterns are stable under a variety of conditions and have been observed as deep as 200 m. We show, using a basic optical model and with experimental measurements, that it is possible to use these patterns, without direct observation of the sun or sky through the water's surface, to perform both local and long-distance navigation tasks. That is to say that an animal with binocular polarization-sensitive vision could maintain a heading relative to the sun over short distances and time-scales; and with additional information (magnetic heading and time) one can determine global location.

[P221] Neural organization of the lamina of mantis shrimp

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Stomatopods, or mantis shrimp, have the most complex retinas in the world. Their compound eyes each consist of three parts: two hemispheres divided by a specialized midband. The midband consists of six rows of ommatidia (single visual units), and the first four rows contain up to twelve colour receptors with narrow spectral sensitivity curves. However, behavioural tests have shown that the spectral discrimination abilities of stomatopods are surprisingly poor, suggesting that they use a different kind of post-processing than the classical opponent comparison system described in other animals. The way that colour information is processed in stomatopods is poorly understood. The first optic neuropil, the lamina, is the first processing station allowing a comparison between different spectral types of photoreceptors. Therefore, studying the lamina is the first step in understanding how mantis shrimp process colour information. The different types of neurons in the lamina of stomatopods have been described anatomically, but it is still unknown how they are connected. Different parts of the retina may also have different patterns of connections in the lamina. This study aims at determining the synaptic connections within the lamina cartridges of the midband rows one to four for each photoreceptor type using light microscopy to describe the overall structures, and serial block-face SEM (3-view) to distinguish synaptic connections.

[P222] Vision and bioluminescence: the evolution of light detection in ocular and bioluminescent organs in deep-sea crustaceans

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Bioluminescence is estimated to have evolved independently more than 50 times across animal taxa. Many organisms use bioluminescence for communication, foraging, and defense, especially in the ocean, where ambient light is faint blue down-welling light from the sun. In clear water, sunlight can penetrate to 1000 m. This research tests hypotheses regarding the evolution of bioluminescence and unique visual systems in Oplophoridae, a family of deep-sea shrimp. Oplophorid shrimp are capable of producing two forms of bioluminescence; an oral secretion to deter or distract predators and cuticular photophores, which are light-emitting organs used for camouflage. Transcriptomic data reveal genes associated with vision (e.g., opsins, arrestins, G-proteins) expressed in photophores of four oplophorid species, and we obtained preliminary evidence of opsin mRNA localization in the photoreceptors in the retina and photocyte nuclei in photophores of Janicella spinicauda. Our preliminary data provides the exciting possibility that photophores "see" light and by working in conjunction with their visual system, oplophorids can actively tune luminance to match down-welling sunlight. Autogenic bioluminescence is found in representatives from every animal phyla on earth so our discoveries will reach far beyond this model system. Furthermore, this research will provide insight into why certain lineages of deep-sea organisms have expanded their visual systems to adapt to life in the dark.

[P223] Switching from stochastic to deterministic patterning in fly retinas: mechanisms and behavioral significance

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In many sensory systems, developmental patterning produces random spatial distributions of sensory receptors. This holds true for most flies, where two different color-sensitive ommatidial types are distributed stochastically across the compound eye. In contrast, some flies in the family of Dolichopodidae (long-legged flies, or "Doli") have retinas, which display a deterministic pattern of alternating columns that appear as vertical stripes. A major step in understanding the functional significance of this striped retina is a detailed characterization of its structure. Using SBFSEM (serial block face scanning electron microscopy) and immunohistochemistry we characterized photoreceptor morphology and Rhodopsin expression in the Doli eye to build a 3D retina model. The orientation of polarization-sensitive rhabdomeres changes in every other row, which corresponds to differential Rhodopsin expression in alternating columns. The conspicuous vertical orientation of the columns suggests that they are aligned with the wave of differentiation that crosses the eye imaginal disc from posterior to anterior during development. We present evidence that the striped pattern of Rhodopsin expression is controlled by the same regulatory network as described in flies.
Future work is aimed at identifying visual stimuli to which the Doli eye has adapted. Comparing behavioral responses of species with deterministic or stochastic patterning will provide insight into the evolutionary significance of retinal stripes.

[P224] Do miniaturization and diurnality account for retinal specializations in the eyes of pumpkin toadlets (Anura: Brachycephalidae)?

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Recent studies have shown that the visual world of anuran amphibians can be quite sophisticated, featuring abilities such as fluorescence emission and colour discrimination in extremely dim light. However, hardly anything is known about the variability of visual systems across different amphibian clades and how it might relate to their diversity of life habits. We are thus working on a comparative study of the family Brachycephalidae, a relatively small clade with two genera: the diurnal Brachycephalus and the nocturnal Ischnocnema. We are studying the composition of the retina at the topographical and ultrastructural levels using light and transmission electron microscopy, and photoreceptor spectral sensitivities using micro-spectrophotometry. In the photoreceptor layer, the most remarkable differences lie in the relative size and shape of the rod photoreceptors and the peak spectral sensitivities. On the other hand, our samples show clear regional differences in the topography of retinal layers in both genera that could be indicative of specializations of the visual field. We will discuss the patterns that these specializations show in relation to phylogeny, body size and diel activity pattern, their potential functional significance, and how future studies should be approached to gain a better understanding of the visual abilities linked to these retinal feature.

[P225] The when and where of stomatopod visual decision-making: advances towards understanding the neuroanatomy and electrophysiology of Pancrustacean brains

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Shallow water stomatopod crustaceans live in dynamic visual environments where they must process behaviorally relevant visual stimuli despite chromatic light flicker and other visual noise. Despite this variability, they can track prey-size objects with their eyestalks, often moving them independently. The central complex, an unpaired midline brain region common to all Pancrustaceans, is implicated in coordinating both independent and conjoint movements due to converging inputs into it from the visual neuropils underlying each compound eye. Here, we present methodological advances in recording extracellularly from the central complex and other brain regions using dual dye channel glass electrodes during visual stimulation. The method allows the localization of the recording electrode by means of a fluorescent dye (Texas Red), marking the site of the electrode tip. The second shaft of the electrode holds a highly mobile fluorescent dye (Lucifer Yellow) that is concurrently released and which enters neurons damaged by the electrode tip, providing information about the projections and neuropil origins of neurons in the immediate vicinity of the electrode. Stomatopods are visually stimulated using a customized LCD display employing technology originally intended for computer gaming enthusiasts. It is used to display depolarized visual stimuli which avoid tearing due to a hardware lock of the video card to the refresh rate of the display at >144Hz. These methodological advances will lead to a better understanding of how action selection of the eyestalks is controlled. In other words, when (correlation of spike times to stimuli), where (recording sites), and to where (projecting neurons).

[P226] How differences in experimental designs can yield support or refutation of the receptor noise model predictions about colour discrimination

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Colour vision, the mechanism through which organisms can detect and process different wavelengths of light, brings viewers important information which is often crucial for survival. The Vorobyev-Osorio receptor noise model estimates the limits of colour or luminance discrimination. This model needs to be tested with behavioural data in order to validate its predictions. We performed two colour choice studies using female guppies (Poecilia reticulata) and compared the receptor noise-limited model predictions with the actual performance of the fish. In the first test, individuals were asked to discriminate a coloured stimulus against an achromatic background. In the second one, females were trained to memorise a coloured stimulus and were asked to find it when presented against a chromatic distractor stimulus. In the first study, most of the behavioural detection thresholds were successfully predicted by the RNL model, while in the second one we found significant discrepancies between the predictions and the behavioural performance. We suggest that while individuals are actually discriminating colour against the background in the first experiment, they are categorising them at a higher threshold in the following experiment.

[P227] High resolution of colour vision, but low contrast sensitivity in a diurnal raptor

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Animals are thought to use achromatic signals to detect small (or distant) objects and chromatic signals for large (or close) objects. While some studies have assessed the spatial resolution of the achromatic channel, the spatial resolution of the chromatic channel has rarely been estimated. In times where ecologists use models to describe what animals can and cannot see, understanding the resolution of both the chromatic and achromatic channel is particularly relevant. Using an operant conditioning method, we estimated (1) the achromatic contrast sensitivity function and (2) the spatial resolution of the chromatic channel of a diurnal raptor, the Harris's hawk Parabuteo unicinctus. The maximal spatial resolution for achromatic gratings was 62.3 c/deg, but the contrast sensitivity was relatively low (10.8-12.7). The maximal spatial resolution of the chromatic channel was 21.6 c/deg - lower than that of the achromatic channel, but the highest found to date in the animal kingdom. Our study revealed that Harris's hawks have high spatial resolving power for both achromatic and chromatic vision, suggesting the importance of colour vision for foraging and detecting prey. By contrast, similar to other bird species, Harris's Hawks have low contrast sensitivity suggesting a trade-off with spatial resolution.

[P228] Signal or cue: the role of structural colouration in flower evolution

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Flowering plants have often evolved pigment colours to visually communicate with hymenopteran insects possessing phylogenetically conserved trichromatic visual systems. Whilst many plant flowers typically achieve colouration using pigments, recent work claims that flowers also produce angle dependent, structural based colours that have evolved for visual communication. These claims are underpinned by appetitive-aversive differential conditioning in lab conditions where bees acquire a capacity to discriminate such colours. In natural environments, however, a pollinator must have the capacity to reliably identify rewarding flowers when considering a variety of approach angles to enable the level of flower constancy required to drive the evolution of signals for effective visual communication between plants and pollinating insects. We used a linearised digital imaging system to record both diffuse and angle-dependent colouration of several plant species of the same families for which angle-dependent structural colouration has been recently reported. Image information was subsequently used to create salience controlled stimuli representing a target flower producing diffuse and angle-dependent colouration. Individual free-flying honeybees were given absolute conditioning to the target stimuli and subsequently tested in transfer tests to understand what information was used to identify flowers as expected for a visual signal. We found no evidence that structural colouration is a signal for bee pollinators, and we discuss possible evidence that flower petal structures that do produce apparent structural colouration are a side effect of nano and microtextured surfaces allowing petals to maintain hydrophilic properties.

[P229] Context-dependent continuous colour discrimination functions help bees to cope with naturally occurring perceptual noise

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An enduring goal of sensory physiology and neuroethology is to understand how animals make decisions based on colour information. Currently employed methods assume that colour discrimination is assured once chromatic differences between stimuli is above a critical threshold imposed by physiological assumptions, although few animals have been behaviourally tested to confirm this position. Using the behavioural colour discrimination data of free-flying bees from four closely related trichromatic hymenopteran species: Apis mellifera, Bombus terrestris, Trigona spinipes, and Tetragonula carbonaria; we reveal that the probability of discriminating colour is not a discrete process, but described by a continuous, sigmoidal-type function which directly links the probability of discrimination with an arbitrarily selected measurement of colour difference. This result indicates that the relationship between colour distance and the success of particular colour discrimination task is mainly mediated by higher level neural processing mechanisms in the bee brain. This complex relationship can be modelled by colour discrimination sigmoidal functions of varying shape. We explore the implications of such a tolerance by discussing the potential problems imposed by the perceptual noise arising from colour variability in two naturally occurring plant species, and a genetically modified plant raised under greenhouse conditions. We further consider this problem in the context of two main models of insect vision (Apis mellifera and Bombus terrestris), showing how perceptual plasticity may help insect pollinators to maintain flower constancy in the presence of perceptual noise.

[P230] From genotype to phenotype for color-based mate choice preferences in Heliconius butterflies

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White or yellow wing coloration in the cydno clade of Heliconius butterflies is a Mendelian trait that correlates with male mate preferences. Homozygotes preferentially court females of the same color, while heterozygotes show no preference. A genome wide association test first identified senseless2 as a candidate mate choice gene that is genetically coupled to the known color locus, is differentially expressed in white and yellow butterflies, and has a potential role in eye development. This finding led us to test the hypothesis that senseless2 expression affects the spectral sensitivity of the eye. Eyeshine experiments indirectly examining the distribution of UV and blue photoreceptors showed variability across species for males but not females. This sexual dimorphism points towards a role in a behavior such as mate choice, but no relationship with wing color was observed. Next we asked whether UV photoreceptors express UV rhodopsin or a Heliconius specific violet rhodopsin that is derived from a UV gene duplication. Electrophysiology experiments showed that photoreceptors were tuned to UV for females and white-preferring males, but tuned to violet for males with yellow or no preference. However, qPCR showed the ratio of UV to violet mRNA varies substantially across species and individuals, suggesting receptor co-expression that could affect UV photoreceptor tuning width and mate choice. Overall, our data are consistent with the hypothesis that senseless2 influences mate choice behavior by altering the spectral sensitivity of the male eye, but additional changes to central brain circuitry are likely necessary to fully explain the behavioral phenotype.

[P231] Shifting cichlid color vision: the role of Tbx2a in LWS opsin gene regulation

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Differences in sensory tuning have been reported to maintain species boundaries, and may even lead to speciation. The tuning of color vision varies dramatically across the 500+ species of African cichlid fishes in lake Malawi. Such variation in color vision is due to differences in the expression of the opsin genes. All species possess seven opsin genes, yet express only a set of three, with sets differing across species. Shifting expression sets requires turning on one opsin while turning off another; for example, species either express LWS or RH2B but not both. The evolution of such shifts are expected to arise through either (a) two simultaneous regulatory changes (one for each opsin), or (b) one regulatory change that simultaneously promotes expression of one opsin while repressing another. Here we use QTL analyses, genome sequencing, and gene expression to identify the transcription factor Tbx2a as a likely candidate promoting LWS opsin gene expression. Binding sites for Tbx2a in the highly conservation Locus Control Regions of both the LWS and RH2B opsins suggest Tbx2a is acting to simultaneously promote LWS expression while blocking RH2B expression. We identify a single deletion upstream of the Tbx2a gene that occurred when a transposable element left this region. Our data support the hypothesis that shifts in opsin gene expression sets are the result of single changes to regulatory sequence.

[P232] Visual communication in cichlid fishes: Do visual sensitivities drive color signals or do color signals drive visual sensitivities

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Sensory drive has been proposed as a key step in the evolution of new species. This process involves feedback between signals and sensory systems as mediated by the environment. For visual communication, visual sensitivities and color patterns might co-vary to optimize communication depending on the environmental light. However, there are a number of different visual tasks that might set the path of sensory evolution with each suggesting different optimal color signals. Further, because it is a feedback loop, it may be difficult to determine if vision sets the evolution of color signals or color signals set the evolution of vision. Here we use visual modeling of African cichlid fishes performing a variety of contrast and color vision tasks as might be important for foraging, predator avoidance, and mate choice. We explore a trichromatic visual system where double cones contribute to contrast and all cones contribute to color vision. By exploring all possible visual systems, we identify the visual systems that are best for particular tasks. We then compare optimal visual systems to actual visual systems. This analysis suggests several results: 1) Visual sensitivities evolve to first optimize contrast detection, primarily for dark objects against bright backgrounds. 2) Once double cones are specified for contrast, cones are spaced spectrally to maximize color discrimination. 3) Visual sensitivities are specified for tasks other than mating, with mating coloration subsequently evolving to maximally stimulate the specified visual system. Hence vision shapes color signals rather than the reverse. We describe how this optimization has influenced cichlid visual communication as they replicately speciate in diverse habitats.

[P233] Cichlid color vision and its limits

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Animals vary greatly in color pattern and such patterns are often involved in animal communication. Studying color vision, the capacity of discriminating color regardless of brightness, is essential to unveil the mechanisms that shape coloration patterns, visual system sensitivities and visual signals. Color vision research benefits from an integrative approach by combining data on photoreceptor spectral sensitivities, behavioral experiments and physiological models. Furthermore, visual systems can be physiologically limited by photoreceptor noise, which prevents accurate chromatic discrimination. Thus, the study of color thresholds is necessary to better understand how color vision shapes animal communication.
Cichlids are colorful freshwater fishes whose color patterns are important for species recognition and mate choice. Hence, accurate visual communication is essential for cichlid survival and may drive cichlid speciation. In this study we show the color vision in a cichlid from Lake Malawi: Metriaclima benetos. We combined behavioral experiments through classical conditioning, and 'The photoreceptor noise-limited model' (RNL) in order to estimate the perceptual capabilities of color contrast. Our results suggest that: (1) M. benetos possesses color vision. Fish were more likely to choose the trained over the distracter stimuli, irrespective of brightness. (2) Fish discriminated between spectrally similar color-pairs above a certain color distance threshold. These experiments allowed us to determine how photoreceptor noise affects color discrimination. (3) Light environment impacts color thresholds. Cichlids under a UV–light environment had different color thresholds than fish under normal lab-illumination. These results are useful to inform laboratory behavioral experiments as well as to estimate cichlid's ability to perform key visual tasks in their natural environment.

[P234] Simultaneous spectral stimulation and two-photon neural activity imaging in a Drosophila colour processing neuropile, the medulla

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The fruit fly, Drosophila melanogaster, has emerged as a key model in vision research. Despite extensive characterisation of motion vision, very little is known about how flies process colour information. We have developed a novel setup that will enable us to accurately dissect the different components of the Drosophila visual system responsible for processing colour. Using flies that express neural activity indicators, we can track visual responses to a projected colour stimulus via a two-photon imaging system. Our visual stimulation setup consists of a customised projector system using a monochromator as its light source to produce many colours (narrow bands of light) across the spectrum. The visual stimulus is projected on a specialised screen material that scatters wavelengths of light across the spectrum equally at all locations of the screen, thus enabling presentation of spatially structured stimuli. Furthermore, the calibration process of the irradiance and spectral contents of the visual stimulus has been automated to allow rapid development of a variety of stimuli. A key feature of our setup is the introduction of specialised bandpass optical filters (or combinations thereof) in two separate locations to allow for the presentation of a visual stimulus with minimal detection of light resulting from the stimulus by the microscope gallium arsenide phosphide (GaAsP) detectors. Using this setup, we are characterising the spectral sensitivity of visual cells in the early visual system of a variety of genetically modified strains of Drosophila.

[P235] Spectral sensitivity of Drosophila melanogaster colour receptors

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The spectral sensitivity of many insect visual systems has been characterised, but few studies have determined the spectral response from tiny insects and even fewer studies have explored the underlying processing of spectral information. Visual pigments, fundamental for colour vision, are responsible for absorbing light and initiating phototransduction. This triggers neural activity and ultimately, a behavioural response. The spectral sensitivities of Drosophila visual pigments are difficult to characterise in vivo using sharp-electrode electrophysiology, due to the small size of photoreceptors and the stochastic expression patterns of colour receptors across the retina. To better understand visual processing in Drosophila, we have firstly characterised the wavelength sensitivities of the photoreceptors and secondly, explored the spectral interaction between different photoreceptor responses, as a potential indication of colour-opponent processing. We have genetically manipulated the natural expression of visual pigments and photoreceptor genes and used the electroretinogram (ERG) method to isolate the spectral responses of individual photoreceptor types. In particular we have used red-eyed flies with a norpA null mutation rendering all photoreceptors non-functional and rescued specific receptor types with opsin promotors controlling norpA cDNA expression. This has enabled the responses of single or multiple photoreceptor types to be obtained. We report the first in vivo spectral sensitivity profiles for the four classes of Drosophila photoreceptors (R7p, R7y, R8p & R8y). Our findings reveal UV sensitivity in the blue-sensitive R8p cells and a significant deviation in spectral sensitivity of the long wavelength-sensitive R8y photoreceptor, from that previously reported.

[P236] The use of spectral cues for orientation in the monarch butterfly Danaus plexippus

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Each fall millions of monarch butterflies (Danaus plexippus) migrate over thousands of kilometers from North America southwards to their overwintering habitat in Central Mexico. To maintain their migratory direction over this enormous distance, these butterflies rely on celestial cues, such as the sun and polarized skylight, as orientation references [Mouritsen and Frost, PNAS (2002); Reppert et al., Curr Biol (2004); Stalleicken et al., JEB (2005)]. In addition, a non-uniform distribution of longer and shorter wavelengths of light generates a skylight spectral gradient that could potentially be used as a compass cue by butterflies during navigation. Here, we asked whether we can test non-migrating butterflies under laboratory conditions and if they can use spectral cues for orientation. The headings of butterflies were tested individually while the animals were tethered at the center of a flight simulator. We presented spectral cues (green and/or UV light spots) to the animals while they were able to freely change their bearing with respect to the light stimuli. Even though the tested butterflies were in a non-migrating stage, they used the lights (green or UV) to keep a constant heading. When we changed the position of the visual stimuli by 180°, the butterflies changed their flight direction accordingly, suggesting that they use the presented cues for course maintenance. Further investigations will reveal if the butterflies use spectral or intensity information to keep their constant heading. Taken together, our data show that non-migrating monarch butterflies maintain a stable direction under laboratory conditions with respect to different spectral cues.

[P237] Psychophysics of the dronefly Eristalis tenax L.: Evidence of sigmoidal colour discrimination capabilities in an important Dipteran pollinator

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The hoverfly Eristalis tenax is a Batesian mimic of the honeybee and is commonly known as the 'dronefly'. The hoverfly is an important pollinator of many flowering plant species and has been implicated as a potential pollinator of agricultural crops. Currently, however, our understanding of fly colour perception in plant-pollinator contexts is based upon models of how the blowfly Lucilia sp. was conditioned to rewarded monochromatic stimuli. This model implies relatively simple visual sense in Dipteran insects which enables the distinct perception of just four different colour categories.To behaviourally measure the biologically relevant colour perception of hoverflies we thus used brightness matched broadband stimuli previously employed to test colour perception in different flower-visiting hymenopteran species. Hoverflies were trained with appetitive absolute conditioning to either a salient 'blue' or a 'yellow' stimuli. In subsequent non-rewarded behavioural tests, we evaluated the choices of hoverflies to perceptually similar graduated steps of coloured broadband cards. Our results show that within proposed categories for Dipteran insect colour vision, flower-visiting hoverflies do not choose between similar 'blue' nor similar 'yellow' stimuli in a manner consistent with a categorical perception of colours. Indeed, the analyses showed some 'blue' and some 'yellow' stimuli are more frequently chosen when they are chromatically similar to the initial conditional stimuli, whilst the frequency of choices diminishes as stimuli became dissimilar to the conditional stimuli. Our findings for Eristalis tenax are thus consistent with continuous, sigmoidal functions describing colour discrimination in flower-visiting hymenopterans, rather than being restricted to categorical colours.

[P238] How seeing red influences food detection: visual ecology in damselfish (Pomacentridae)

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Damselfish (Pomacentridae) occur on temperate to tropical reefs around the world and show a great diversity in colouration and ecology. Feeding specialization such as herbivory or planktivory, have been shown to be linked to differences in visual sensitivities in these fishes. Differences in the light absorbance of visual pigments (opsins) determine the spectral sensitivities of an organism and shape its visual ability. Molecular studies on opsin genes suggest that herbivorous damsels feeding on algae which reflect in the far-red have red shifted visual systems when compared to zooplanktivorous species that feed on UV absorbing crustaceans. In this study the putative correlation of seeing red and being herbivorous is investigated in detail. Spectral sensitivities are measured using microspectrophotometry and incorporated in theoretical damselfish visual models. These models are then used to design behavioural assays to investigate spectral discrimination abilities in one species-pair of herbivorous versus planktivorous damselfish. Elucidating how the visual phenotype is linked to a fish's lifestyle is key to unravel the role vision may play in shaping the complexity and diversity of coral reef ecosystems. Ultimately, this study will help to better understand the crucial role herbivorous fishes play in sustaining a healthy reef, knowledge that is urgently needed in the light of recent declines of coral reefs worldwide due to human induced climate change.

[P239] Quantitative Colour Pattern Analysis (QCPA): A Novel Approach for the Study of Animal Colour Patterns

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To understand the design and evolution of animal colouration it is essential to quantify animal colour patterns and how they appear against their natural backgrounds as seen by ecologically relevant viewers. We have recently made significant progress to overcome quantitative and qualitative limitations of current spatiochromatic colour pattern analyses by combining calibrated digital photography, visual modelling, behavioural experiments and pattern analysis into a novel analytical framework, called the Quantitative Colour Pattern Analysis (QCPA). QCPA provides a powerful platform for quantitative and qualitative in-situ colour pattern and visual contrast analysis in both aquatic and terrestrial environments.
QCPA allows us to investigate key hypotheses on the function, design and evolution of defensive animal colouration, particularly crypsis and aposematism. We apply QCPA to investigate colour patterns in nudibranch molluscs, an intriguing and understudied model system for the study of defensive animal colouration and eco-toxicology in the marine environment. Using nudibranch molluscs as a model system I will show how QCPA can be used to address signal honesty, mimicry, design principles shaping the conspicuousness of nudibranch colouration as well as intra and inter specific variation of colour pattern geometry in the context of phylogenetic data and geographic distribution. Furthermore, I will discuss how QCPA can also be used to calibrate behavioural experiments with complex visual backgrounds and stimuli.

[P240] Colour discrimination thresholds throughout colour space in the reef fish Rhinecanthus aculeatus

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To predict how a coloured stimulus, such as camouflaged prey, appears to another species, researchers rely on behavioural experiments and visual models. Colour discrimination has previously been investigated in a variety of species, however, due to the constraints of traditional methods, thresholds are only known for a few sets of colours and limited areas of colour space. This is an important gap in current knowledge because research from human psychophysics suggests that discrimination thresholds vary according to the direction of the colour change and the area of colour space tested. We used a novel method to determine the discrimination thresholds of a reef fish, Rhinecanthus aculeatus, throughout colour space. We employed printed stimuli similar to Ishihara colour plates used to identify colour blindness in humans. Fish were taught to find an odd coloured spot on a background of distractor spots and peck it to receive a food reward. We conducted three experiments, which tested discrimination thresholds on increasingly saturated backgrounds. Our results indicate that colour discrimination thresholds vary throughout colour space and according to the direction of the colour change. Additionally, our results suggest that estimates of receptor noise based on relative abundance underestimate noise in the short wavelength receptor in this species. Our method offers a new means to examine colour vision in a variety of species and may be adopted to investigate novel research questions. Our results emphasise the importance of behaviourally calibrating visual models and indicate that discrimination thresholds are highly variable throughout colour space.

[P241] Anatomical evidence for ultraviolet vision in larval stomatopod crustaceans

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Adult stomatopod crustaceans are well-studied and known for having arguably the most complex visual system in the world. In contrast, there are limited studies of the larval eye, which is physiologically and morphologically distinct from the adult eye. There remains a gap in understanding of the function and development of the larval visual system. It is generally assumed that all pelagic larval crustaceans contain similar compound eyes with a single spectral class of photoreceptor, regardless of adult eye complexity. We hypothesize that stomatopod larval eyes are more complex than previously believed and contain a second ultraviolet (UV) photoreceptor, making them more similar to adult crustacean retinas. Stomatopod larvae are planktonic and live in pelagic ocean environments where UV light is known to penetrate up to several hundred meters. Planktonic species are hypothesized to use UV light for a variety of tasks, including aiding in vertical migration for crustaceans and detection of prey for planktivorous larval fish. The hypothesis that UV photoreceptors are present in stomatopod larvae was tested through investigation of the ultrastructure of the larval eye. Preliminary transmission electron microscopy (TEM) images of a stomatopod larval eye display evidence of an extra cell in the photoreceptor, predicted to be analogous to the R8 photoreceptor in adult stomatopods responsible for UV vision. If R8 cells are present in stomatopod larval retinas, this will be among the first evidence that stomatopod larvae have UV sensitivity. Follow up studies will be needed to understand the ecological function of UV vision for stomatopod larvae.

[P242] A model explaining the colour interaction in the receptive fields of the ganglion cells in the goldfish retina

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Three types of cones (long-wave, middle-wave and short-wave sensitive) in the goldfish retina determine its color vision observed in its behavior. However the most of retinal ganglion cells (GCs) projecting to the tectum opticum – motion detectors – seem to be colour blind. Our electrophysiological experiments with cone-isolated visual stimulation of different spectral types of cones demonstrated that these GCs are connected with all three types of cones. Simultaneous stimulation of two types of cones reveals complex interaction of colour channels which sometimes is difficult for description, and the retinal circuitry underlying this interaction looks unclear and needs to be modelled. The model is made in the form of a three-layer artificial neural network. An input layer consists of three receptors corresponding to three spectral cone types of the goldfish. Neurons of the second layer (bipolar cells) are connected with receptors by adjustable synaptic connections. The neuron of the third layer (a GC) is connected with second layer neurons by non-inverting rectifying synaptic contacts. The weights and signs of the synapses of the second layer neurons are adjusted by the researcher to get the responses to color stimulation similar to those observed in the experiments. There is no unambiguous relationship between the constructed network and the real scheme of retinal cell connections. Nevertheless the model permits to combine the experimental data on the interaction of color channels in the receptive fields of the GCs of different types and to imagine the number of neuronal units necessary for organizing such an interaction. Supported by the RFBR grant 16-04-00029

[P243] Fluorescence emission from photonic structures in beetles' elytra

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Fluorescence emission occurs in the integuments of many natural species including but not limited to insects, arachnids, mammals, anthozoans (e.g., sea anemones and corals) and scyphozoans (i.e., true jellyfish). In arthropods, fluorescent molecules, such as papiliochrom II and biopterin, are at the origin of such light emission. In some cases, they are naturally embedded in photonic structures, which influence the emission in terms of spectral intensity and spatial distribution[1-2]. Using different microscopy (i.e., light, electron and fluorescence) and spectroscopy (i.e., spectrophotometry and spectrofluorimetry) techniques, the cases of fluorescent beetles with different types of photonic structures (quasi-ordered photonic crystals and randomly-disordered structures) were investigated. These structures control both the colourations of these insects and the emissions of the embedded fluorophores. In addition to optical simulations, such observations allow to study the photonic confinement within the beetles' structures. Fluorophores contained within one species were chemically characterised by liquid chromatography-mass spectrometry. Additionally, Third-Harmonic Generation and two-photon fluorescence analyses performed on selected species unveiled the multi-excited states character of the fluorophores and, through light polarisation effects, the role of the photonic structures' anisotropy in the fluorescent behaviour. In addition to the elaboration of new concepts and the development of technological applications through a bioinspiration approach, such investigations help understanding the biological functionalities behind the observed fluorescence response. [1] P. Vukusic et al. Directionally Controlled Fluorescence Emission in Butterflies, Science (2005), 310, 1151. [2] S. R. Mouchet et al. Controlled fluorescence in a beetle's photonic structure and its sensitivity to environmentally induced changes, Proc. R. Soc. B (2016), 283, 20162334.

[P244] How to be camouflaged against multiple visual backgrounds

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Arguably the most common form of anti-predator defence in nature is camouflage. This can be achieved via many routes, from resembling specific objects (masquerade), matching the general visual environment (background matching), to breaking up tell-tale features such as the body outline with high contrast markings (disruptive coloration). Most work to date has focussed heavily on how camouflage is best achieved on one background type alone. However, in reality, animals (and human-made objects) will move around between backgrounds and visual environments also vary from one patch to another. This means that in many cases matching one background closely will not be optimal. While some theoretical work has explored how 'compromise camouflage' can overcome this problem, it remains relatively unexplored. In addition, it has been suggested that disruptive coloration, because of how it breaks up body shape, may work even with a lack of background matching. Here, we conduct a series of experiments with human subjects searching for controlled hidden artificial prey on computer touch screens. We demonstrate to what extent compromise camouflage and disruptive coloration are effective in preventing detection when an object occurs over a wide range of visual environments, as opposed to specialist background matching. Our results have implications for the evolution and form of both natural and applied camouflage.

[P246] Limits for a beetle using polarized light in the dark-a psychophysical study.

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Insects are known to incorporate information from the sky to maintain their headings, using patterns of brightness, colour and polarization to set and hold their course. Nocturnal dung beetle Scarabaeus satyrus can use the dim lunar polarization pattern to roll its dung ball in a straight line, much as its diurnal relatives use the solar polarization pattern. While for clear nights this pattern has a stable and predictable configuration, it varies drastically in both brightness and degree of polarization as a function of time of night and moon phase. It has also been demonstrated that the degree of polarization of lunar skylight can be reduced by anthropogenic light pollution, levels of which rise year-on-year. We developed a darkroom behavioural paradigm to investigate the effects of degree of polarization and brightness on orientation precision in S. satyrus, determining which conditions would allow for accurate orientation. We present these data in the context of polarimetric sky imaging to estimate the quality of lunar skylight available to the beetles in their natural habitat across different moon phases.

[P247] First-order ocellar interneurons in the posterior protocerebrum of the night-active bee Megalopta

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Megalopta is a genus of night-active bees from Central and South America. Their extremely sensitive apposition compound eyes [1] allow them to navigate their dim jungle habitat [2]. Additionally, Megalopta have three large dorsal ocelli of unknown function. The ocellar photoreceptors are slow, green sensitive (peak at 500 nm), and have no recorded responses to UV or polarised light [3]. Contrary to the photoreceptors, little is known about the ocellar interneurons that transmit information to the brain. Based on intracellular recordings combined with 3D reconstruction, we found four broad anatomical categories of these neurons: 1) Cells innervating only one ocellus (lateral or median) and terminating in the posterior protocerebrum (PP); 2) Cells connecting one lateral ocellus to the thoracic ganglia with minor branching in the PP; 3) Cells innervating one lateral and the median ocellus and branching in the PP and the suboesophageal ganglion (SOG); and 4) Cells innervating either one lateral or all three ocelli and projecting to the medulla of the optic lobe. All these cell types responded similarly to the presence of light by hyperpolarisation and/or inhibition of action potential firing. We also found cells with persistent responses to UV light, suggesting UV sensitive ocellar photoreceptors exist in Megalopta (opposed to earlier findings [3]), or that interactions between the compound eye and ocelli take place.
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[2] Stone et al. (2017) Curr. Biol. 27 (20), 3069-3085.
[3] Berry et al. (2011) J. Exp. Biol. 214 (8), 1283-1293.

[P248] Action in dim light: vision and visual navigation of nocturnal ants

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Ants, despite their tiny size, relatively small brains and few neurons, are highly competent visual navigators. Several ant species restrict their activity to brightly lit periods during the day where visual information is reliable. A significant number of ants, however, are active in dimly lit environments, including animals that forage in the dark confines of the leaf-litter, in closed canopy rain forests, or at night. In dim-light habitats, the visual signal-to-noise ratio is typically low, which makes detecting reliable visual navigational information a challenge. Here, we will identify the behavioural, optical, and physiological adaptations that ants have evolved for being efficient visual navigators in dim-light.

[P249] Optimising vision in twilight conditions: photoreceptor transmutation in the deep-sea pearlside

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Most vertebrates have a duplex retina comprising two photoreceptor types, rods for dim-light (scotopic) vision, and cones for bright-light (photopic) and color vision. Deep-sea fishes, however, are only active in dim-light conditions hence, most species have lost their cones in favour of a simplex retina composed exclusively of rods. While pearlsides, Maurolicus spp., appear to possess such a pure rod retina, their behaviour is at odd with this simplex visual system. Contrary to other deep-sea fishes, pearlsides are mostly active during dusk and dawn close to the surface, where light levels are intermediate (twilight or mesopic) and may require the use of both rods and cone photoreceptors. To explore this paradox, we investigated the visual system of two pearlside species. Results show that their previously categorised all-rod retina is in fact composed almost exclusively of transmuted cone photoreceptors. In other words, the pearlside does not possess the usual rods and/or cones but instead possess a third type of photoreceptor. These transmuted cells combine the morphological characteristics of a rod photoreceptor with a cone opsin and a cone phototransduction cascade to form a unique photoreceptor type, a rod-like cone, specifically tuned to the light conditions of the pearlsides' habitat (blue-shifted light at mesopic intensities). This is the first report of photoreceptor transmutation mediating a cone-opsin-based visual system in teleost fishes. These results challenge the standing paradigm of the vertebrate duplex retina and its evolution, and call for more comprehensive evaluations of visual systems in general.

[P251] Active photolocation in diurnal fishes: Can they see more by redirecting downwelling light?

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Diurnal active photolocation (DAP) is the process by which diurnal fish re-direct downwelling light into the horizontal visual plane using their own irides to generate reflections in cryptic target organisms, facilitating their detection. Although nocturnal active photolocation is known from chemiluminescent fishes, diurnal active photolocation is a controversial hypothesis. This debate follows from unfavourable conditions during the day (bright backgrounds) and a lack of relevant data. Our research focuses on quantifying the components of this process, and testing predictions experimentally. This allows us to narrow down the morphological, perceptual, and ecological niche in which DAP may function. Important properties that will affect the contribution DAP can make to target detection are (1) the presence of highly reflective eyes in targets, (2) iris radiance and modulation in the observer, (3) distance between sender and target, (4) small-scale light gradients, and (5) the receiver's visual system. The key question is whether real-world combinations of these properties can result in functional DAP. Our work focuses on a single species, the triplefin T. delaisi, a small (3-5 cm), crypto-benthic micro-predator [1]. We present unpublished data from visual modelling and manipulative experiments that support the idea that DAP can supplement visual detection of cryptic predators in an ecologically relevant part of the parameter space. Given the ubiquity of small benthic species with specialised forms of iris radiance and reflective eyes in many prey and predator species, this is likely to be a widespread phenomenon. [1] Michiels, N. K., et al. (2017) Controlled Iris Radiance in a diurnal Fish looking at Prey. Royal Society Open Science.

[P252] Daytime eyeshine contributes to pupil camouflage in a cryptobenthic marine fish

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Ocular reflectors enhance eye sensitivity in dim light, but can produce reflected eyeshine when illuminated. Most teleost fish occlude their reflectors during the day. The opposite is observed in cryptic sit-and-wait predators such as scorpionfish and toadfish, where reflectors are occluded at night and exposed during the day. This results in daytime eyeshine, proposed to enhance pupil camouflage by reducing the contrast between the otherwise black pupil and the surrounding tissue. We test this hypothesis in the scorpionfish Scorpaena porcus and show that daytime eyeshine is the result of two mechanisms: the already known Stratum Argenteum Reflected (SAR) eyeshine, and the Pigment Epithelium Transmitted (PET) eyeshine, a newly described mechanism for this

species. After confirming that the ocular reflector of the scorpionfish is exposed when the eye is light-adapted, we measure the relative contribution of SAR and PET eyeshine to pupil brightness and show that these estimates can be used to reliably predict field data. Finally, visual models for different light scenarios in the field show that daytime eyeshine contributes to pupil concealment from the perspective of a prey fish. We propose that the reversed occlusion mechanism featured by some cryptobenthic marine predators has evolved as a compromise between camouflage and vision.

[P253] Single object resolution in budgerigars (melopsittacus undulatus)

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Visual acuity of birds is traditionally measured as the ability of the eye to resolve high contrast gratings. Behavioural experiments testing grating acuity generally agree quite well with the anatomical measurements of ganglion cell density, indicating that sampling density of the retina is the limiting factor of spatial acuity. However, in an ecological context it can also be relevant to know how small single objects an eye can resolve. For example, in what distance is a foraging bird able to spot a seed or an insect on the ground, or to detect a flying bird of prey against the sky? The budgerigar (Melopsittacus undulatus) has been used extensively as a model organism in the study of avian vision. Their contrast sensitivity and visual acuity for gratings has been determined in behavioural experiments (Lind & Kelber, 2011, J Vis 11(7):2) and acuity also anatomically by retinal ganglion cell density (Mitkus et al, 2014, J Comp Physiol A 200:371–384).
Now we have also examined the limit for visual resolution of single objects in budgerigars. Using a two-choice behavioural procedure we trained birds to distinguish circular dark objects presented on a screen. We used objects of different size and contrast to the background as well as objects with sharp or blurred edges to see how these parameters influenced the perception of the birds. Our results indicate that the ability of the budgerigars to resolve single round objects (ca. 7.5 cycles/degree) is well below that for gratings (ca. 10 cycles/degree).

[P254] Frequency tagging identifies selectively attended target in a dragonfly visual neuron

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The visual world projects a complex and rapidly changing image on to the retina, which is a computational challenge for an animal relying on an accurate view of the world for survival. One such challenge is parsing a visual scene for the most salient targets for goal-directed behaviour, such as the selection of prey amidst a swarm of potential targets and conspecifics. Previously we identified a dragonfly visual neuron that exhibits selective attention when presented with multiple, equally salient features, called 'Centrifugal Small Target Motion Detector 1' (CSTMD1). We perform intracellular electrophysiological recordings from CSTMD1 neurons in vivo, whilst presenting visual stimuli on a monitor display. In order to identify the target selected in any given trial, we modulated the intensity of moving targets, each with a unique sinusoidal frequency (frequency-tagging). We find that the frequency information of the selected stimulus is preserved in the neuronal response, whilst the distracter is completely ignored. We show that the attentional system that underlies selection in this neuron is can be biased by the presentation of a preceding target on the same trajectory. With an improved method of identifying and biasing target selection in CSTMD1, the dragonfly provides an effective animal model system to probe the mechanisms underlying neuronal selective attention.

[P255] Specialised Vision, Target Selection and Control in a Miniature Robberfly

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The miniature robber fly Holcocephala fusca achieves an impressive visual acuity of ~0.28° and a visual detection threshold of ~0.12 °, but object detection is only the first challenge of a predatory attack. Killer flies and dragonflies have all been shown to use heuristic size/speed rules for deciding target size and range suitability, but mantids use stereopsis. Here we have investigated with three different experiments whether Holcocephala's target choice is explained by heuristic rules or stereopsis. Experiment 1: we presented the animals with a choice targets of different sizes and velocities, which moved at a constant velocity. Experiment 2: targets followed a circular path, and therefore their angular size and velocity were constant across the trial. Experiment 3: we removed any velocity cues by covering the bead with a mirror (which reflected the open sky) during positioning. The results of all three experiments are consistent. Target choice by Holcocephala is not explained by a loose fitting subtended size/ angular speed ratio, or by any other relative visual cue. Hence, despite a paucity of relevant cues, Holcocephala demonstrates an ability to distinguish absolute target size. After taking off, Holcocephala displays a range of different behaviours, either aborting the flight before hitting the target, shadowing it at a fixed range or directly intercepting it. During interception, Holcocephala operates a reactive proportional navigation controller, like those used in modern missiles, under a reaction delay of ~28ms. Holcocephala also uses a control gain of 3, demonstrated to use minimal energy expenditure on steering responses.

[P256] A Robber Fly with Similar Gleaning Habits, but Very Different Eyes to Damselflies.

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Damselflies (Zygoptera) are able to pluck spiders from their web, a classic example of gleaning predation. Some Leptogastrinae flies, here named darting robber flies, also use this strategy. For example, Psilonyx annulatus is a darting robber fly which hunts in the shaded undergrowth, gleaning stationary prey from the vegetation. Given the similarity between damselfly and darting robber fly predation, it is surprising that their eyes reflect opposing adaptations; while damselfly eyes are conspicuously separated, the foveas of P. annulatus are located right next to the midline. This is especially intriguing because the body of P. annulatus resembles that of damselflies. To elucidate if the specifics of the gleaning behaviour explain the eye difference, we have reconstructed P. annulatus's predatory attacks in 3D. Upon detection of prey, P. annulatus turns towards it and delivers a three-phased attack. In the first phase (approach), the predator slowly flies closer to its prey. In the second phase (bobbing), the fly draws a loopy trajectory around a central point, maintaining a set speed. During the third phase (surge), the metathoracic tibiae flex, while the fly accelerates rapidly in a straight line towards the target. The attack fails if the prey starts moving during the surge. To our knowledge, the bobbing behaviour here reported is absent in damselfly attacks. Therefore, we suggest that the placement of the foveas in P. annulatus minimises ocular disparity, and that the animal uses motion parallax from the bobbing behaviour to ascertain prey suitability, which damselflies would presumably do at once via stereopsis.

[P257] Binocular Facilitation in Damselfly Target Selective Descending Neurons

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Dragonflies and damselflies both perform complex visually guided aerial manoeuvres for prey capture, albeit with distinct visual adaptations and hunting strategies. For instance, the dragonfly species well-studied to date track and attack their prey from below using a single fused (holotopic) dorsal fovea. In contrast, damselflies attack their prey in a frontal assault, using their frontward-facing foveas and conspicuously separated eyes. At the core of these divergent behaviours is a sensorimotor transformation. In dragonflies, a small set of 16 bilaterally symmetric Target Selective Descending Neurons (TSDNs) efficiently encode prey position and direction of movement as a population vector. TSDNs are thought to stabilise the prey within the dorsal fovea for interception. Thus, TSDNs serve as an ideal handle to investigate the divergence of sensorimotor transformation in these animals. We have identified and recorded from TSDNs of the Banded Demoiselle (Calopteryx splendens). Calopteryx's TSDNs show directional and spatial tuning properties for object movement reminiscent of dragonfly TSDNs. Although the receptive fields in Calopteryx also tend to be centred at the midline, they usually expand across both hemifields. Our binocular and monocular inputs experiments show that Calopteryx's TSDNs receive binocular input, and that at least 2 types of TSDNs only respond when both eyes are stimulated at the same time, i.e. binocular facilitation. Binocular facilitation of this type is present in cats and monkey neurons highly sensitive to disparity, called "binocular only neurons". This is the first report of a "binocular only" neuron in an insect, whose mechanism we are currently investigating.

[P258] A case of mistaken identity? Linking attack behaviour and visual perception in sharks.

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Shark bites on humans, although infrequent, can have devastating impacts on the victims and their communities. The level of fear engendered by these infrequent events is exacerbated by a lack of understanding with respect to the reasons why they occur. One theory behind shark bites on humans is that of mistaken identity, whereby sharks are thought to mistake humans for their typical prey (pinnipeds in the case of white sharks, Carcharodon carcharias). This study addresses the plausibility of the mistaken identity theory by comparing video footage of pinnipeds and humans swimming, and humans paddling surfboards, from the perspective of a shark viewing these objects from below. Video clips were filtered to reflect how the shark's visual system would perceive the visual motion and shape cues. The shark visual system was modelled using data from electrophysiological, microspectrophotometric and anatomical studies. Visual motion cues were analysed using a 2-dimensional motion detector model. Object similarity was calculated using a distance metric based on the position between the centroid and the perceived edges of the object. Our modelling suggests that, humans swimming or paddling surfboards may appear quite similar to pinnipeds from the perspective of a shark, which may lead to unintended bites to humans.

[P259] Neuronal pathways of the lateral protocerebrum of mantis shrimps

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Mantis shrimps (stomatopods) are known for their aggressive strikes and their unparalleled visual system containing 16 different photoreceptors and the ability to discern linearly and circularly polarised light. Stomatopods are a good study model to understand how a simple brain is used to process a complex visual system. Similar to most other eumalacostracans, a stomatopod eyestalk contains a lamina, medulla, lobula, and a collection of smaller neuropils termed the lateral protocerebrum (LP). These neuropils are clusters of interneurons that receive, process and relay information, which eventually reaches the central brain via the optic nerve. This study aims to investigate the LP by describing its fine neuroanatomy, synaptic connections, and major pathways. Mass fills using Dextran Texas Red fluorescent dyes have been applied to understand how the lamina, medulla, lobula, and the central brain are connected to the LP, and this information will be used to create a neuronal road map of the extensive network in the LP. Initial results revealed numerous tracks between the LP and the central brain. The majority of the tracks are confined to the center of the LP, except for a prominent axon bundle which passes the medulla and branches off into the hemiellipsoid body, a prominent neuropil in the LP, and notable smaller bundles that feed into the lobula.

[P260] Changes in the velocity of an approaching object are tracked by a locust motion-sensitive visual interneuron

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Locusta Migratoria is an established neuroethological system for the study of sensory coding within a well-defined motion-sensitive visual neural pathway consisting of two identified interneurons, the Lobula Giant Movement Detector (LGMD) and its postsynaptic partner, the Descending Contralateral Movement Detector (DCMD). The DCMD connects to thoracic interneurons and motor neurons and is implicated in initiating and coordinating avoidance behaviours. This pathway responds robustly to visual motion that occurs in the locust's natural environment. It is most sensitive to motion of objects approaching on a collision course and can also track changes in object trajectory and motion of multiple objects within the visual field. Data also suggest that this pathway responds to objects that change approach velocity, which could be encountered during predator attacks. We recorded from the DCMD while presenting locusts with looming stimuli that increased or decreased in speed during approach. The change in velocity occurred within a behaviourally-relevant time window. We presented stimuli against simple (white) or flow field backgrounds. Results show that the DCMD responds to a velocity decrease with decreased firing rate approximately 50 ms after transition and the post transition peak is reduced compared to the pre-transition peak. Following transition to a higher velocity, the response is masked by ongoing firing rate modulation but is consistent with responses to a constant faster approach. The presence of a low field minimally affected DCMD response across stimuli. These results suggest that this motion-sensitive pathway is capable of actively adapting to objects that alter their velocity during approach.

[P261] Seeing the world in a different light – visual processing of intensity and polarization to enhance target detection

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Many crustaceans are sensitive to the polarization of light across their whole visual field. They use polarization cues and signals as important forms of visual information for object-based visually guided behaviours. The use of polarization in functional tasks that require parsing of visual scenes suggests that crustaceans could process polarization information in ways that enhance visual contrast across the image-forming eye. While there is some evidence to suggest that polarization and intensity information may be integrated into a single contrast channel whereby polarization directly modulates perceived intensity, this single contrast model has never been explicitly tested. Therefore, we tested the predictions of this model using a series of visual psychophysics experiments in which we determined the behavioural response probabilities of the fiddler crab Uca tangeri to different looming stimuli. The polarization and intensity properties of these stimuli were adjusted independently and simultaneously using a novel type of visual display technology. We show that crabs do not integrate polarization and intensity information into a single contrast channel as previously suggested, but instead process information relating to these two modalities in discrete channels. This discovery disproves the previous hypothesis that the motion vision pathway sensitive to looming cues in crabs, and perhaps in other crustaceans, perceives polarization as variations in intensity. Instead, intensity and polarization both contribute to their own measure of contrast in early visual processing of looming cues, which then feeds into processing circuits that mediate target detection and visually-guided behaviour.

[P262] Visual priming within a modular visual system

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For over a century it has been known that jumping spiders (Salticidae) use vision to a level unprecedented in other groups of spiders, and the visual system and associated visual behaviour of salticids has, since this time, been of considerable interest to researchers. Like other spiders, salticids have eyes that fall into two anatomically and ontogenetically distinct categories: primary and secondary. Classic work on the salticid visual system has focused primarily on the unique characteristics of their primary eyes. Using a specialised eye-tracker to probe the underlying characteristics of salticid vision, we examined the information-processing role of the secondary eyes by presenting visual stimuli to the spider secondary and primary eyes while recording the response of the primary eye retinae. The secondary eyes were primed with a stimulus (circle or bar) that either matched or mismatched a stimulus later presented to the primary eyes. Analyses of the scanning patterns and scanning activity of the primary eyes indicates that visual priming is occurring within the salticid visual system. This is the first evidence of visual priming happening within the distributed visual system of a salticid. By facilitating the scanning patterns of the primary eyes, priming from the secondary eyes is likely to cause salticids to make faster and more accurate decisions in nature.

[P263] Coding Schemes in the Archerfish Optic Tectum

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Most of what is known today about how the early visual system works is based on studies of terrestrial animals. Although these works have yielded valuable data on the function of early visual processing, they are biased by the fact that they have focused mostly on terrestrial mammals. To better account for visual systems in different environments and animal classes, we studied the structure of early visual processing in the archerfish which harnesses its extreme visual ability to hunt by shooting water jets at prey hanging on vegetation above the water. Thus the archerfish provides a unique opportunity to study visual processing in a vertebrate which is an expert vision-guided predator with a very different brain structure than mammals. The receptive field structures in the archerfish optic tectum, the main visual processing region in the fish brain, were measured and linear non-linear cascades were used to analyze their properties. The findings indicate that the spatial receptive field structures lie on a continuum between circular and elliptical shapes. In addition, the cells' functional properties display a richness of response characteristics, since many cells could be captured by more than a single linear filter. Finally, the non-linear response functions that link linear filters and neuronal responses were found to be similar to the non-linear functions of models that describe terrestrial mammalian single cell activity. Overall our results help to better understand the early visual processing system across vertebrates.

[P264] Visual circuits underlying the prey capture strike in zebrafish larvae

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Zebrafish larvae rely on their visual system to hunt and capture prey objects, such as paramecia. Prey capture begins with a series of stereotyped orienting turns and swims that allow the larva to approach the prey, followed by a more propulsive swim coupled with jaw opening to create suction and consume the paramecium. The orienting phases of hunting are evoked by small moving objects. However, the stimulus for the final capture strike remains unknown. We have developed a behavioral paradigm to elicit and classify strikes in head-fixed larvae. Here we present the assay and results of initial experiments to define the stimulus. Going forward, we will use this preparation to shed light on the visual circuitry underlying the strike.

[P265] A cholinergic pesticide impairs contrast sensitivity and direction tuning in hoverfly motion detecting neurons

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Cholinergic pesticides such as the neonicotinoid imidacloprid (IMI) are widely used chemicals for crop protection against specific pest species, but also have unwanted effects on beneficial insect species, such as pollinators. Neonicotinoids act agonistically on nicotinic acetylcholine receptors (nAChRs) at synapses that are widespread in the insect nervous system. Intensive recent investigation on effects of sub-lethal doses of IMI in bees revealed (among others) impaired navigation, homing and foraging behaviours. However, although insects strongly rely on visual cues to forage and navigate their environment, the neurophysiological effects of IMI on the insect visual system have been largely overlooked. To fill this gap, we focused on the well-known population of motion sensitive, direction-selective lobula plate tangential cells (LPTCs) in the hoverfly, Eristalis tenax. These cells are activated by ACh, possibly through the presence of nAchRs. We monitored LPTC activity when the brain of the insect was perfused with sub-lethal doses of IMI or its vehicle (dimethyl sulfoxide, DMSO). We recorded from LPTCs while stimulating the eye with different directions of grating stimuli and variable contrast. We found that IMI increased the spontaneous activity, while also decreasing both directional selectivity and contrast sensitivity compared to either the normal condition or neurons exposed to DMSO. Our results reveal new evidence for the neurophysiological effect of IMI in an intact pollinator and suggest a possible mechanism by which cholinergic pesticides might act in the brain of flying insects to affect motion-guided navigation.

[P266] Differential adaptation to visual motion allows robust encoding of optic flow in the dragonfly

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An important task for any aerial creature is the ability to ascertain their movement through their environment. This behaviour has been well established in many insect models including flies, moths and bees, but little research has been conducted in dragonflies. Interestingly, certain species of Dragonflies (e.g. Hemicordulia Tau) engage in hawking behaviour, hovering in a single area for extended periods of time whilst also engaging in highly dynamic pursuit and fast-moving patrolling behaviours. These different behaviours place very different constraints on establishing ego-motion from optic flow. We have identified several classes of widefield-motion sensitive neurons in H. tau via intracellular electrophysiological recording and anatomical staining. The classes exhibit distinguishing features in both their anatomy and physiology. Anatomically, some of these cells are more complex than their fly counterparts with multiple arborisations in different lobes of the lobula neuropil. Physiologically, these neurons exhibit similar spatial and temporal frequency tuning properties in their initial responses to motion, but differ markedly in their adaptation properties and response to the broadband statistics of natural images. These different motion adaptation properties are well matched to the specialised flight behaviours found in dragonflies. We also find differences in the cells' direction opponency with some cells responding to motion in all directions whilst others are strongly inhibited by anti-preferred motion. Altogether, these findings match well with the exquisite flight behaviours of dragonflies and may help explain the complex neuronal architectures which underlie dragonfly flight.

[P267] Neural summation improves motion vision in small fruit flies

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For holometabolous insects like the fruit fly, limited larval feeding, common in nature, results in smaller adult flies. Despite the importance of vision to flies, smaller adults possess smaller eyes that sacrifice contrast sensitivity due to smaller optics. During forward optic flow, images in the periphery move faster than in the central field of view. Many flying insects have modified optics in the lateral regions of their eye, such as an increased inter-ommatidial angle for greater light capture. The fruit fly, however, has a nearly homogeneous eye structure but improves peripheral reliability by spatially summating dynamically in the lateral eye. Do smaller-eyed conspecifics modify this neural summation strategy given their sacrifices in optics? To address this, we generated a broad distribution of eye sizes by removing larva from their food during their third instar and measured their visual abilities in a virtual flight arena. In the central field of view, small adults maintain spatial acuity by sacrificing contrast sensitivity at the optical level, but recover contrast sensitivity through temporal summation at the neural level. However, temporal summation would only exacerbate motion blur in the periphery during forward optic flow. Instead, in the periphery, smaller eyes neither temporally nor spatially summate, sacrificing contrast sensitivity in the lateral eye. In combination with optic flow-induced, regionally specific spatial summation, regionally specific temporal summation in smaller eyes minimizes the sacrifices made by having smaller optics. This neural plasticity allows small-eyed conspecifics to compete visually with their larger counterparts, even at high flight speeds.

[P268] Systematic identification of neurons in the brain of Drosophila – Neurons with projections in posterior neuropils to ocellar ganglion interneurons

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The posterior brain of Drosophila contains projections of at least 49 descending interneurons, which connect the brain with the ventral nerve cord (Namiki et al., 2017). Therefore, posterior neuropils are important centers for descending commands to initiate or maintain specific motor programs. Signals from the ocelli, secondary visual organs located on top of the head of many flying insects, are also received by posterior brain neuropils and are known to modulate motor activity (e.g. Mizunami, 1994). As yet, we do not have detailed information about the neuronal circuit architecture in posterior neuropils and how neurons convey visual information from the ocelli to the brain. We screened several thousand Drosophila GAL4 expression driver lines to identify neurons with projections in the ocellar ganglion (OCG) and in the posterior neuropils: the Antler (ATL), Inferior Bridge (IB), and Superior Posterior Slope (SPS). By using the split-GAL4 intersectional strategy, we were able to generate stable fly lines whose expression patterns are specific to neuron types of interest. We also used the multi-color flip-out technique to provide detailed information about single cell morphology.
Our screen for ATL, IB, and SPS revealed at least 245 specific neuron types, 93 of which have projections in both the IB and SPS. Furthermore, we identified eight OCG interneuron types of three major classes: Local

interneurons, with projections only in the OCG, OCG output neurons, and OCG input neurons. Our results provide a detailed neuroanatomical map of the interconnections between the ATL, IB, SPS, OCG and other neuropils to deepen our understanding of the Drosophila brain.

[P269] The dominant role of visual motion cues in bumbleMark flight control revealed through virtual reality

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Previous studies have established that flying bees make extensive use of optic flow: the apparent motion in the visual scene generated by their own movement. However, the use of real physical objects in such studies limits the scope for experimentally manipulating cues. Here we implement a virtual reality system allowing us to create the visual illusion of objects in 3D space. We trained bumblebees, Bombus ignitus, to feed from a static target displayed on the floor of a flight arena, and then observed their responses to various interposing virtual objects. When a virtual floor was presented above the physical floor, bees were reluctant to descend through it, indicating that they perceived the virtual floor as a real surface. To reach a target at ground level, they flew though a hole in a virtual surface above the ground, and around an elevated virtual platform, despite receiving no reward for avoiding the virtual obstacles. These behaviours persisted even when the target was made (unrealistically) visible through the obstructing object. Finally, we challenged the bees with physically impossible ambiguous stimuli, which give conflicting motion and occlusion cues. In such cases, they behaved in accordance with the motion information, seemingly ignoring occlusion

[P270] Response of a locust motion sensitive neuron, flight muscle activity and wing asymmetry during flight steering

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Flying animals display a variety of adaptive behaviours to avoid predators and collisions with conspecifics during flight. The locust Descending Contralateral Movement Detector (DCMD) is a well characterized motion-sensitive visual neuron that responds with an increased firing rate that peaks near the time of collision (TOC) of an approaching object. Increasing stimulus complexity (number and shape of objects or object trajectory changes) affects the amplitude and temporal properties of the DCMD response profile. This is the first experiment to examine DCMD responses during flight steering. Preliminary data show that, compared to a non-flying condition, DCMD in flying locusts responded to a head-on approach (0°) with a narrower response profile and lower peak firing rate whereas an approach from 45° evoked a later peak. Previously described DCMD bursting occurred in non-flying and flying locusts, suggesting that bursting is critical for coding object approach. Bursts also showed an earlier increase in intraburst firing rate during a 45° loom, compared to an approach from 0°. This indicates that bursting responses change with respect to stimulus direction and that bursts are an important component of coding to coordinate motor output during collision avoidance behaviour. EMGs from forewing left and right steering depressor muscles (m97) revealed earlier Lm97 activation, indicating a left turn, when presented with an object approaching from the right. High speed video of the forewings demonstrated wing asymmetry in which the left wing depressed ahead of the right wing. These results provide insights into general principles of coordinated flight steering.

[P271] Visual motion detection and collision avoidance behaviours are disrupted by a neonicotinoid insecticide and its metabolites in Locusta migratoria

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Neonicotinoid insecticides are used extensively in agriculture in North America, despite known environmental impacts. These insecticides affect foraging behaviour and navigation in non-target insects, although the mechanisms of these effects are not fully understood. A visual motion sensitive neuron in the locust, the Descending Contralateral Movement Detector (DCMD), integrates visual information and is involved in eliciting escape behaviours. The DCMD receives coded input from the compound eyes and synapses with motorneurons involved in flight and jumping. We show that imidacloprid (IMD), a neonicotinoid insecticide, impairs neural and behavioural responses to visual stimuli at sublethal concentrations, and these effects are sustained two and twenty-four hours after treatment. Exposure to sublethal doses of IMD attenuates escape manoeuvers and disrupts DCMD bursting, a coding property important for visual motion detection. Furthermore, we show these effects can be elicited by one of the metabolites of IMD, 5-hydroxy-imidacloprid, at lower concentrations than the parent compound. This metabolite is produced in invertebrates and plants, and we show for the first time that it decreases visual motion detection at very low concentrations. Overall, we confirm significant and lasting impairment of an important pathway involved with visual sensory coding and escape behaviours. These results show, for the first time, that a neonicotinoid insecticide directly impairs an important, taxonomically conserved motion-sensitive visual network.

[P272] The response properties of visual interneurons in the mantis unravel the functional organization of the lobula complex

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The praying mantis shows broad repertories of visually guided behaviours such as prey recognition and defence to collision. However, the neural basis of them has been poorly understood. Visual system of the mantis comprises retina and three neuropils, lamina, medulla, and lobula complex. Recently, it has been reported that the lobula complex in the mantis consists of five neuropils, outer lobe 1 and 2 (OLO1 and OLO2), anterior lobe (ALO), dorsal lobe, (DLO), and stalk lobe (SLO). However, functional organization of lobula complex remains unclear. In the present study, we studied the response properties of neurons in these neuropils by intracellular recording and staining. Some ALO neurons and DLO neurons responded to large-field motion such as drifting gratings and showed directional selectivity, suggesting the possibility that ALO and DLO might correspond to lobula plate in other insects. OLO and SLO neurons responded to a small moving target with highest spike firing. Some of them ramified both sides of optic lobes, suggesting their possible role in stereopsis. Some ALO neurons best responded to looming stimuli like lobula giant movement detector (LGMD) in locusts. Possible functional organization of lobula complex in the mantis will be discussed.

[P273] Adaptation in the visual motion pathway shapes representation of optic flow in aerial insects

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For volant insects, the retinal image motion (optic flow) elicited by their flight within an environment not only contains information about their self-motion, but also about the spatial layout of the environment with ecologically relevant objects. In insects, optic flow is processed in successive neuropile layers. The neurons in these layers share adaptive response features (i) to compensate for the limited operating range of neurons, (ii) to limit energy costs in generating neuronal responses, (iii) to provide a stable sensing platform even when physical parameters in nature vary widely, and (iv) for robustness against external and internal noise. While adaptation alters the response gain to the signal, some information needs to be retained consistent for behavioral control. By systematically modeling the adaptive motion pathway of the insect visual system based on electrophysiological data on adaptation at different processing layers of blowflies, and simulating the visual input during flight in virtual and natural 3D environments, we could analyze the impact of adaptation on signal representation under various environmental conditions and flight dynamics. We find that brightness adaptation is essential for robust spatial vision under dynamic environmental conditions. Motion adaptation further enhances foreground-background segregation. Our studies on adaptation in population coding of global flow fields may give insights on what information is important to maintain for downstream neurons. Given the general similarity in the mechanisms of motion computation in a wide range of animal groups, our conclusions are not restricted to the model system of this study.

[P274] Responses to visual motion stimuli of lobula giant neurons from a crab assessed by multielectrode recording

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One of the main challenges in neuroscience nowadays is to understand the concerted function of individual neurons dedicated to particular behaviors in the behaving animal. This goal first requires to attain an adequate characterization of the behavior as well as an identification of the key neuronal elements associated to that action. Such conditions has been considerably attained for the escape response to visual stimuli in the crab Neohelice. In fact, a combination of in vivo intracellular recording and staining, with behavioral experiments and modeling, led us to postulate that a microcircuit formed by four classes of identified lobula giant (LG) neurons operates as a decision making node for a number of important visually-guided components of the crab´s escape behavior (1). These studies, however, were done by recording LG neurons individually. In order to investigate the concerted functioning of the LG group, we began to use multielectrode extracellular recordings. Here we describe the methodology and show results of simultaneously recorded responses from different neurons to a variety of visual stimuli. The different LG classes can be distinguished by their electrical activity and differential responses to visual stimuli. Simultaneous recordings confirmed the rightfulness of previous interpretations about LG interactions assumed from independent intracellular recordings. The current results establish the bases for and show the feasibility of our next goal of recording the activity of LG neurons in the behaving animal. (1) Tomsic D. Visual motion processing subserving behavior in crabs. Curr. Op. Neurobiol. 41: 113-121. 2016

[P275] OFF pathway specializations in the Drosophila visual system

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In many sensory systems including the visual system of Drosophila melanogaster, there are distinct ON and OFF pathways. In Drosophila, these pathways split postsynaptic to photoreceptors, where the two first-order interneurons L2 and L3 are the major inputs to circuitry that detects OFF edge motion, whereas the ON pathway receives input from the L1 neurons. The functional role of the distinct OFF pathways is not known. Here, we show that the physiological properties of the L2 and L3 input neurons are very different. When we use in vivo two photon imaging to record calcium signals in L2 or L3 axon terminals in response to prolonged light stimuli, L2 responses are transient, whereas L3 calcium signals are sustained. Using a range of visual stimuli we could show that L2 is contrast sensitive as it provides downstream circuits with information about recent changes in luminance, whereas L3 is luminance sensitive and responds strongest at low luminance. Thus, the two cells in the OFF pathway respond to fundamentally different features of the visual scene. To understand these early differences in visual processing, we tested the contribution of different photoreceptors inputs, lateral circuit inputs and downstream feedback mechanisms. Together, our data argue that lamina neuron properties are shaped by cell autonomous mechanisms and are not a result of circuit interactions. We are currently working towards identifying the mechanisms that shape the distinct physiological properties, as well as probing the specific behavioral roles that these two OFF pathways play in motion detection.

[P276] Making sense of directions: The complete set of input cells to the upper division of the central body in bees

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The central complex (CX) is a group of highly conserved neuropils localized in the center of the insect brain. It consists of the protocerebral bridge (PB), the upper and lower divisions of central body (CBU, CBL) and the paired noduli. Its regular neuroarchitecture of vertical columns and horizontal layers is generated by a multitude of columnar and tangential neurons. Certain sets of PB columnar neurons serve as head-direction cells in flies, while homologous neurons in locusts are tuned to celestial polarized light. In both species the CX thus serves as internal compass. Compass information reaches the CX via CBL-tangential neurons, and an interplay between the CBL and the PB likely generates the array of head-direction cells. In bees, we recently proposed a model in which CX-output cells combine head-direction information with path integration memory stored in the CBU for guiding the insect back to its nest. This model predicts that CX-mediated steering could be based on any information relayed to the CBU. Yet it is unknown which information reaches this structure from other brain areas. To delineate these pathways we comprehensively characterized the input neurons to the CBU (TU-neurons) in the bumble bee. We performed 3D-tracing of the CBU-branches of all TU-cells in one individual bee, based on block-face electronmicroscopy of the CX. The partial TU-cell morphologies yielded this way were complemented by individual, but completely traced TU-cells obtained from intracellular dye-injections. Together, this detailed description of CBU-input neurons now provides the basis for physiological studies that will illuminate how insects use their head-direction code for making navigational decisions.

[P277] The Bogong moth: A new model for visually-guided long-distance navigation

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The Australian Bogong moth (Agrotis infusa) is known for its remarkable ability to precisely navigate under low-light conditions: During their spring migration, these nocturnal moths fly over 1000 km to reach their estivation sites in the Australian alps, before returning to their breeding grounds by the same route in autumn. This yearly migration event has become iconic in Australia; yet our understanding of how these small moths navigate so precisely remains poor. Behavioural experiments on tethered flying moths suggest that Bogong moths require visual cues, such as landmarks and optic flow, for maintaining oriented flight. Furthermore, recent behavioural data showed that the moths can sense the geomagnetic field and possibly use it to select a flight direction. By mapping the anatomy of the Bogong moth brain using immunohistochemistry, we can show that the same neural substrate that is involved in compass navigation in other insects is also present in the Bogong moth. Intracellular sharp-electrode recordings from these brain areas – most notably the central complex and lateral accessory lobes – reveal neurons responding to visual compass cues as well as neurons responding to optic flow. This provides the foundation for more complex stimulation protocols, additionally involving magnetic fields, to simulate conditions during migratory flights. Taken together, the Bogong moth is a fascinating new insect model for visually-guided long-distance navigation that offers the opportunity to reveal the neural underpinnings of the brain's compass and steering system not only with respect to a celestial visual compass, but possibly also a magnetic compass.

[P278] Phototactic flight of the chestnut tiger butterfly is based on the dorsal eye region

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The compound eye of butterfly species consists of three distinct regions that can be distinguished by their anatomical features: dorsal rim, dorsal, and ventral. The dorsal eye region is best placed to extract directional cues such as spectral or intensity gradients from the sky pattern. In this study, we explore the function of the dorsal eye region in the migratory chestnut tiger butterfly, as this animal presumably uses aerial visual cues to guide long distance migration. We released a butterfly in a small cage illuminated with fluorescent tubes and four halogen lamps. The butterfly spontaneously flew near the ceiling of the cage. When two additional solar lamps were introduced at one side of the cage, the butterfly's flight became biased towards them. This phototaxis persisted when the solar lamps were blue-filtered and greatly reduced in intensity. However, when yellow filters were used, the phototaxis was eliminated. We repeated the experiment using butterflies whose eyes were partially covered with paint, in order to establish the eye regions involved. Phototaxis behavior of individuals with either their very dorsal region or the ventral two thirds of the eye occluded was broadly similar to that of intact individuals, indicating that the dorsal region is sufficient for phototaxis. However, butterflies with the dorsal third of their eye covered would not fly, instead perching on the cage wall. These results indicate that phototaxis in the chestnut tiger butterfly depends on the spectral composition of light, and is likely mediated by the dorsal eye region.

[P279] Correlation between stimulus information and escape behaviour in fiddler crabs

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Escape responses are fundamental and ubiquitous components of animal behaviour. Their robust and reproducible structure can provide important insights into how information is integrated and processed by nervous systems. In this context, invertebrates provide ideal study systems because their low spatial resolution allows realistic simulation of predators with simple computer-generated stimuli. In the laboratory, escape responses are commonly triggered using looming stimuli, which have been used extensively in many species, including the crab Neohelice granulata, to examine escape timing. In fiddler crabs (Uca spp), we have a detailed understanding of what information elicits escape responses in the field. In the laboratory, however, the correlations between visual information and escape behaviour have not yet been fully explored. Here we focus on the visually guided escape behaviours shown by the fiddler crab Uca dampieri, on a treadmill and an artificial mudflat environment. Responses are elicited by a variety of 'predator' simulations including pure looming, and looming stimuli that also have a translational component. Initial findings show strong differences in the elements of escape behaviours between the two stimuli, such as running or freezing, suggesting that perceived threat levels are different between the two stimuli. Additionally, the speed of pure looming stimuli strongly affected the timing and probability of escape. This is congruent with field results in the same species, but different to results obtained from other crabs. The results of this study will allow us to explore the effects laboratory constraints have on animals and to understand better the importance of context in escape-related decision making.

[P280] Human and conspecific face discrimination abilities of cats and dogs: learning, recall, and preferences

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Dogs and cats have been domesticated for thousands of years and have emerged as the most popular pets in North America, with nearly two-thirds of all households having at least one. Most cat or dog owners have a deep bond with their animal companion and believe that their dog or cat can actually recognize their face. But is this true? It is likely that there are a number of factors cats and dogs use to discriminate different humans; including facial features, frequency and pattern of speech, olfactory signals, and differences in movement or gait. The question posed by the present study was, "In the absence of acoustic, olfactory, and movement cues, do dogs or cats prefer the face of their handler over the face of a stranger?" To accomplish this we tested twelve beagles and twelve domestic cats to determine if they would preferentially choose the face of their human handler and the faces of other familiar animals. Dogs had a profound preference for familiar human or familiar conspecific faces, while cats only showed a preference for a familiar conspecific face. Neither cats nor dogs demonstrated a preference when presented with pairs of unfamiliar human or unfamiliar conspecific faces. Our results show that dogs strongly prefer the face of their handler over the face of similar, unfamiliar humans. This suggests that dogs use facial characteristics as a factor in determining the identity of their handler. Presumably these skills have evolved as a consequence of prolonged human interaction following domestication.

[P281] Paying attention to polarization: What cues do mantis shrimp learn most quickly?

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Mantis shrimp (stomatopoda) are unusual in how much they rotate their eyes around the axis of view. Unlike other animals, these eye rotations are not involved in image stabilization, but intermittently transform the visual coordinates of the outside world on the retina of the animal. Evidence suggests that one of the functions of these rotational eye movements is related to polarization vision: mantis shrimps rotate their eyes to optimize the polarization contrast in an image, roughly analogous to a photographer rotating a Polaroid filter on a camera to improve the image. A possible consequence of this arrangement is that the animal may discard information about the absolute angle of polarization (AoP), which changes constantly as the eye rotates, and opt instead to pay attention to differences in the amount (or degree) of polarization (DoP). To investigate this, pairs of peacock mantis shrimp (Odontodactylus scyllarus) were trained with a food reward to discriminate between light sources varying in either AoP or DoP. The rate at which the animal learned the task was used as a proxy for information content of the cue. To ensure that animals received the same testing routine and to avoid experimenter bias, trials were performed using an automated system, which controlled stimulus presentation, detected mantis shrimp choice behavior, and delivered food rewards when appropriate to two animals in parallel. The findings provide insight into the information content of polarization cues and will shape our approach to understanding the high-level processing of polarization information in the complex visual system of the mantis shrimp.

[P282] Miniaturisation in ants affects their vision and visual navigation

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Evolution of extremely small body size in a lineage, reduction beyond which is not possible is a phenomenon called miniaturisation. It has dramatic effects on an animal's ecology, anatomy, morphology, and physiology. Miniaturisation has captivated biologists imagination because tissues and organs reach limits imposed by the physical world, leading to alternative behavioural strategies. But the behavioural cost of miniaturisation has largely remained unexplored. Here we study ants that exhibit dramatic variation in body size where irrespective of size, they all have a unifying behaviour: to find their way back home. We quantify the variation in the number of ommatidia between ants of different sizes. Using pattern electroretinography, we tested whether the reduction in size affects spatial acuity and contrast sensitivity. Ants derive compass information from the pattern of polarised skylight. They detect this through specialised ommatidia located in the dorsal rim region of their eye. We tested whether the number of specialised ommatidia scales with the total number of ommatidia in various ants and whether this relates to the ability of animals to navigate using the pattern of polarised skylight. In addition, we tested how miniaturisation in ants affects navigational abilities such as obstacle avoidance. This poster will provide an overview of how miniaturisation in ants affects vision and visual navigation behaviours.

[P283] Visual navigation in ants: what is the function of the mushroom body?

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Successful navigation is crucial for finding mates, foraging, defending territories, and avoiding predators. While a variety of sensory stimuli can be used for navigation, vision is an important modality in most animals, including many ant species. Even with miniaturized brains, ants are exceptionally accurate at visually navigating and pinpointing locations of interest. The Australian bull ant Myrmecia midas, specifically, is highly visual and primarily uses terrestrial landmarks for finding its way home from a foraging trip. These landmarks are learned early in the ants' foraging career. The behavioural characteristics of learning and using terrestrial landmarks have begun to be described in a few ant species; however, the brain regions and mechanisms involved in processing navigational information are not well understood. We tested the hypothesis that the mushroom body alpha lobe, a region specifically implicated in long-term memory formation, is involved in processing terrestrial landmark information. In M. midas foragers that were motivated to return home, we pharmacologically inhibited activity in the alpha lobe. In this talk I will demonstrate how inhibiting alpha lobe activity affects the ability of ants to visually navigate in their natural environment.

[P284] The long and short of it: Spatial cognition of detours to prey by jumping spiders

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Jumping spiders (Salticidae) of several species, including Portia spp. (Spartaeinae) and Phidippus spp. (Dendryphantinae), have been demonstrated to be able to plan routes to prey that they cannot jump directly on or walk straight toward. Salticid visual acuity is very good and such situations must often occur in nature as the spiders forage among shrubbery, recognize acceptable prey and then calculate a detour route through the vegetation to reduce the separation and bring the spider to a vantage point from which it can jump on to its prey. In laboratory experiments with spiders of Phidippus regius, we examined two aspects of potential spatial cognitive planning that a jumping spider might employ when approaching prey. We used a binary choice protocol to test whether spiders prefer to take the shorter versus longer path to prey when both lead to prey. We tested a range of path disparities. At smaller disparities, spiders chose randomly, but as path disparity increased, the probability of a spider choosing the shorter path increased to more than 70%. Whether the spider judges absolute disparity or uses Weber's Law to judge proportional differences will be discussed. In a second experiment, we offered spiders a choice of two equidistant paths both leading to the prey, but one terminated above the prey and the other terminated at a similar separation below the prey. Twice as many spiders chose the elevated path that would allow them to jump down on the prey.

[P285] Neural basis of sun compass navigation in Drosophila

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Insects exhibit impressive navigational abilities, from long distance migrations of monarch butterflies to path integration of desert ants in the genus Cataglyphis. Although not generally considered migratory, mark-recapture experiments indicate that Drosophila can cover 10 km of open desert in perhaps as little as a few hours without stopping to refuel. This impressive feat required flies to adopt a fairly straight path, likely accomplished by visually guided navigation using celestial cues. Using a flight simulator with machine-vision wing tracking, we found that tethered D. melanogaster can use the position of a simulated sun to fly straight, and individuals adopt arbitrary headings. A preferred heading is maintained over short intervals, but fidelity decays as the time between flights is increased. Furthermore, by restricting visual stimuli to one-half of the arena during flight, we could bias subsequent headings towards the direction of the initial stimulus. Recent advances in our understanding of Drosophila central complex function during navigation reveal that wedge neurons of the ellipsoid body, a homologous structure to the lower division of the central body in other insects, are important for visually-guided locomotion. Using 2-photon functional imaging we found that the activity of these neurons tracks the position of a simulated sun, similar to results for flies responding to other visual objects. Genetic silencing of wedge neurons using the inwardly rectifying potassium channel KiR2.1 appears to restrict the distribution of flies' headings frontally. Future experiments on these and other central complex neurons are likely to reveal neural elements that are highly conserved in insect navigation.

[P286] Visual acuity and behavioral camouflage in the flying snake (Chrysopelea)

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Flying snakes (Chrysopelea) are arboreal species capable of gliding appreciable distances. With large eyes relative to head size, and a large, developed optic tectum, these snakes appear to have visual specializations. In flight, flying snakes can actively control their glides by turning in air. At rest, flying snakes occasionally track objects overhead, including birds and airplanes, presumably to identify predators. This evidence suggests that vision may be integral to both the snake's arboreal and airborne behaviors. They may use vision to estimate the jump distance and timing of in-air turns, inform landing choices, and avoid predation. However, the limitations of their visual system are currently unknown.
This talk will describe flying snakes' behavioral responses to visual motion, and the spatial and temporal limits of their vision, based on a series of experiments measuring optokinetic response (OKR). We find that flying snakes have a large field of view, with a small binocular area. Flying snakes can respond to a large range of temporal frequencies (0.2 – 90 Hz), and have moderate spatial vision, with roll-offs in performance occurring around 20 cpd.
In our experiments, we have also observed head wagging, a lateral oscillation of the head. This behavior often occurs during gap-crossing tasks, which suggests that head wagging serves to help determine distance via motion parallax. However, we have also observed this behavior during OKR experiments, with significant kinematic differences. These differences suggest that head wagging is a contextually dynamic behavior, serving as motion parallax in some visual contexts, and behavioral camouflage in others.

[P287] A vision-based system for avoiding mid-air collisions

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With the relentless increase of air traffic, there is a pressing need for autonomous sense-and-avoid guidance systems that are inexpensive and applicable across a variety of platforms. Nature suggests numerous potential strategies for collision avoidance that exploit simple concepts. In this study, we develop a theory that describes how visual information acquired by each aircraft, can be used to estimate the Time to Minimum Separation (TMS) and the Absolute Minimum Separation (AMS) between two aircraft. Each agent (animal or aircraft) mitigates collision avoidance by modulating its flight speed, without changing flight direction. Our theory prescribes which aircraft should increase or decrease its speed, in order to increase the AMS. It turns out the required modulations of speed are always reciprocal, and mutually beneficial. That is, when aircraft A is required to increase its speed, aircraft B must decrease its speed, and vice versa. Interestingly, each aircraft can use its visual information to determine the required polarity of its speed change independently, without any communication with the other aircraft. This strategy for collision avoidance has been tested extensively in simulations. The attractive features of this strategy are that it is simple and non-cooperative. That is, each aircraft senses and mitigates the collision by acting independently, using its own sensing mechanisms, and without reliance on external infrastructure such as satellite-based GPS. Apart from immediate applications to the aviation industry, this strategy may have relevance in the context of collision avoidance in flying insects and birds, which may be worthy of exploration.

[P288] Budgerigar flight: Guidance laws for avoiding mid-air collisions

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There are more aircraft occupying our sky than ever before. The inexorable increase of air traffic, particularly of Unmanned Aerial Vehicles, underscores the rising need for inexpensive and reliable systems for the automatic avoidance of mid-air collisions. Birds are extremely adept fliers, travelling rapidly through dense natural environments and complex urban landscapes without colliding with obstacles or each other. Nature has seemingly already solved the same problems we are currently facing in the design of unmanned aerial vehicles. How birds achieve these incredible feats, however, is largely unexplored. In the present study, we investigate how birds (Budgerigars) avoid mid-air collisions by seeking to understand how they identify obstacles, and to identify the strategies they use to avoid collisions with them. In a series of experiments, we presented individual flying Budgerigars with three different scenarios: (i) obstacle-free flight; (ii) flight past a static model bird; and (iii) flight past a model bird with flapping wings. All experiments were conducted in a purpose-built flight tunnel and the flights were recorded with four synchronized cameras at 200 fps. The average radial separation profile exhibited by the Budgerigars in the flapping model bird scenario was compared with the predictions of a strategy in which the bird holds the optic flow generated by the model bird constant while flying past it. Our findings so far suggest that Budgerigars veer away from the model bird in such a way that the optic flow generated by the model bird is held constant. This appears to be a simple, vision-based strategy for sensing and avoiding imminent collisions.

[P289] Flying in a 'bee cloud': Mid-air collision avoidance strategies

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When foraging bees are prevented from re-entering their hive, they fly in a holding pattern near the hive entrance. This scenario, which we term a 'bee cloud', offers an excellent opportunity to investigate how a group of insects (bees) avoid mid-air collisions when flying in close proximity to one another in the vicinity of a landing target (hive). Many researchers have shown that the collision avoidance response in insects is exhibited through turning manoeuvres. In this study, we focus on the factors which influence the turning behaviour of the bees in a cloud. Our initial analysis has revealed that the turning flights in a horizontal plane are influenced by the spatial distribution and proximity of other bees in the vicinity. The joint effect of these two factors can be analysed by computing the Integral proximity factor (IPF), which is a combined measure of the number of bees as well as their proximity within a visual field. We calculated the IPF in the left and right visual sectors of bees which were performing horizontal left and right turns. A higher IPF in the right visual sector was observed when the bees were turning left, and vice-versa. Our preliminary findings suggest that bees turn away from the visual field in which the bees are more numerous and/or closer.

[P290] Habituation to looming stimuli in zebrafish larvae

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Habituation, a simple form of learning defined as a diminishment of an innate response to a frequently repeated stimulus, is critical to our everyday focus and attention, and the neural mechanisms underlying habituation remain unclear. Zebrafish larvae show habituation to visual and auditory stimuli, and provide an appealing platform from which to study habituation’s circuit-level mechanisms. When presented with a looming stimulus that resembles an approaching predator, larvae respond with a rapid escape behaviour. In this project, I will first explore whether zebrafish larvae are capable of habituation to repetitive looms, and then use a SPIM microscope and calcium imaging to visualize neuronal activity and localize the regions associated in this learning behaviour. In our behavioural experiments, we use a screen below an arena with freely swimming larval zebrafish. Our results confirm that they habituate visual threats, as they gradually decrease the probability of startle responses during a train of looming stimuli. Neuronal functional imaging experiments with GCaMP6s zebrafish larvae are now helping us uncover the neural circuits and patterns of activity responsible for this habituation. These experiments involve the simultaneous observation of activity across thousands of neurons spanning the entire brain early and late in the habituation process. By identifying the patterns of activity that precede, coincide with, and follow habituation, we aim to gain circuit-level insights into how habituation occurs.

[P291] Face recognition in fish

Dr Ulrike Siebeck[1], Dr. Amira Parker[1], Dr. Cait Newport[2], Prof. Matthias Franz[3], A/Prof. Guy Wallis[1]
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Fast and accurate recognition of individuals is important for animals living in social systems. In some animals such as fish, facial colours and patterns vary between individuals, while in others, such as humans, facial features vary more in the spatial domain. Face recognition and discrimination is thought to require specialised neocortical structures in humans, structures that fish lack. We tested face recognition in two fish species to determine (1) which features Damselfish use to discriminate between conspecific and heterospecific fish and (2) to determine whether Archerfish are able to discriminate human faces despite their lack of a neocortex. In each case, fish were trained to an image of a specific face presented on a computer screen, located in or above their tank. The fish were then tested against another image of a face and their task was to identify the trained image. Ambon damselfish did this by biting the trained image, whereas Archerfish spat at the image displayed on the screen above their aquarium. We found that Ambon damselfish were able to discriminate facial patterns belonging to conspecific fish from those belonging to heterospecific fish and used machine learning techniques to determine the features they used to solve the task. We also found that Archerfish were able to identify a learned facial image when tested against a large set of distractor faces, and even across changes in viewpoint, demonstrating human face recognition in a biologically unprepared animal with no neocortex.

[P292] How Dory finds her friends

Dr Fabio Cortesi[1], Dr Sara Stieb[1,2], Dr Karen Cheney[1], Prof Justin Marshall[1]
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Coral reefs are one of the most diverse and colourful habitats on the planet, and many marine organisms use vision to find food, avoid predation, attract mates and compete for resources. At the core of animal vision are opsin genes, and the evolution of opsins is a textbook example of how changes at a molecular level drive adaptation to divergent photic environments and lifestyles. However, few studies on the evolution of opsin genes in reef fishes exist, but such information is needed to elucidate how fishes adapt to new habitats, utilize novel resources, and may explain adaptive speciation in reef fish families. In this study we investigated the diversity and evolution of opsin genes in surgeonfishes (Acanthuridae). Surgeonfishes are one of the most colourful and species rich fish families found on tropical and temperate reefs. Moreover, surgeonfishes exhibit high morphological and ecological diversity ranging from ‘standard’ fish shapes to scurrile ‘unicorn like’ morphologies. They feed on a variety of sources including organic detritus, planktonic animal matter, invertebrates and algae, and typically experience major ecological changes when transitioning between ontogenetic stages, including resource shifts and changes in gregariousness. Most importantly, surgeonfishes are considered a key group for the health of coral reefs by, for example, preventing algal overgrowth. Hence, understarding their visual ecology and how this drives adaptation to varying environments and lifestyles is crucial for the future mangement of this fish familiy. This is especially urgent given the detrimental impact human activity is having on coral reefs world-wide.

[P293] Seeing on the reef: the visual ecology of the spotted unicornfish (Naso brevirostris)

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Surgeonfishes (Acanthuridae) represent some of the most common herbivorous fishes on tropical coral reefs and both their ecology and behaviour contribute substantially to the health of corals by e.g. limiting algal overgrowth. The spotted unicornfish, Naso brevirostris, is a highly abundant surgeonfish which is known to undergo changes in diet from algae to zooplankton and habitat from coral-associated to pelagic when transitioning from juvenile to adult stages. My Master’s thesis will investigate, at the cellular and gene-expression level, whether an ontogenetic shift is evident in the visual system of wild-caught specimens and whether this putative shift correlates with changes in diet and/or habitat of this species. We hypothesize that there will be a change in the specialization of the visual system which correlates with changes in feeding modes, e.g. a red to UV shifted visual system as diet changes from red-reflecting algae to UV-absorbing plankton. One of the main goals of this study is to characterize the expression of the N. brevirostris visual genes (opsins) and to visualize their locality in the retina. In my investigation, I will use a variety of approaches such as retinal mapping, high-throughput RNA sequencing (RNAseq), in-situ hybridization, Microspectrophotometry (MSP), and histology.

[P294] Diversity of Visuomotor Reflexes Seen in Two Drosophila Species

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In order to generate adaptive behavior, animals must identify salient features in their environment and ignore background noise. A salient feature must also be assigned a positive or negative value. Many insects, including the fruit fly, Drosophila melanogaster, find long vertical stripes attractive, while work by Miamon et al., 2008, described D. melanogaster’s strong aversion to small visual stimuli. This size-dependent value assignment is thought to help D. melanogaster navigate towards potential perching or feeding spots and avoid potential predators or collisions with other insects. Just as changes in local odor environment have been shown to direct olfactory preferences across species of Drosophila, we investigated whether features of varied local environments might similarly drive context-specific valence assignments to visual stimuli. We asked whether the size-correlated value assignments D. melanogaster assign visual objects are conserved across species from varied environments, by examining the desert fly, D. mojavensis. Using a ‘virtual-reality’ flight simulator, we presented D. melanogaster and D. mojavensis with visual objects that D. melanogaster consider attractive (long stripe) or aversive (small box) and used resulting steering behavior as a readout of the value assigned to each object. Like, D. melanogaster, we found that D. mojavensis steer towards long stripes. Interestingly, however, unlike D. melanogaster, D. mojavensis find small objects attractive or of neutral value. These findings reveal a previously unknown diversity of visuomotor processing among Drosophila species and provide a platform for further investigation into the role of local landscape in the development of visual processing circuits.

[P295] Mapping the natural visual world of the zebrafish (Danio rerio)

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Zebrafish (Danio rerio) is a popular model in vision research, but a detailed understanding how their visual system is adapted to their natural environment is still missing. Such a link can help to understand the evolution of vision and provide new perspectives when designing species-specific visual stimuli. By surveying the spectral content of zebrafish's natural environment, we found that chromatic information systematically varies along the vertical axis with increasing depth, resulting in three separate zones with different chromatic features. Zebrafish have 4 cone types sensitive to UV, blue, green and red light. To examine their natural visual world, we used a custom waterproofed hyperspectral scanner to construct full spectrum images from 31 shallow underwater scenes of West Bengal, India. As expected, water and dissolved particles absorb most of the shorter wavelengths of light red-shifting the spectrum available for underwater vision. The resultant peak wavelength aligns near perfectly with red cone sensitivity, leaving the UV cones at the extreme edge with minimal number of photons available. Next, we separated achromatic and chromatic information by principal component analysis. This revealed a chromatically rich horizon with achromatic zones above and below. In the upper visual field shorter wavelengths are still present, while longer wavelengths dominate near the bottom. As shown in Zimmermann, Nevala, Yoshimatsu et al. (2017), these results provide a quantitative and testable series of key predictions for chromatic processing in the zebrafish retina. References
Zimmermann*, Nevala*, Yoshimatsu* et al. 2017. Zebrafish differentially process colour across visual space to match natural scenes. bioRxiv.

[P296] On the role of the visual and vestibular systems in stabilising perching in zebra finches

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Birds have outstanding abilities to balance on two legs and maintain upright posture on both rigid and highly flexible moving perches. However, to our knowledge, there has been no formal study investigating which sensory modalities are used to control upright posture in birds and, more importantly, how these are combined. From observation, we hypothesized that birds use a combination of visual, vestibular and proprioceptive information to balance on a perch. We used a perch torque sensor to measure the torques and forces exerted on the perch under different circumstances to explore this question. To test the degree to which vision contributes to balancing ability, we removed compared perching ability in the light and dark. Zebra finches (Taeniopygia guttata) were assessed for their ability to perch using high speed infrared videography and perch torque measurements in light and dark conditions over several weeks. In the dark perch-torque root-mean-squared deviation from 0 was greater in light conditions (p=0.01), suggesting that birds were more active in light conditions. However, neither spectral analysis of the data nor peak derivatives measures did not indicate that perching ability was significantly affected by changes in light. Ongoing experiments involve damaging vestibular hair cells with amino glycoside antibiotics, to assess the role of the vestibular system in maintaining upright posture while perched.

[P297] Honeybees choose their way to home using e-vector information from the sky.

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Many insects use the polarization pattern of the sky to obtain compass information during navigation. Also in honeybees, it is well known that the waggle dance orientation is drastically affected by the e-vector orientation of the skylight. To investigate how they respond to the polarized light during flights, we constructed a flight simulator for a flying tethered bee, in which the bee was stimulated with zenithal polarized light. When the e-vector orientation of the polarized light stimulus was slowly rotated, the tethered bee showed clear polarotaxis, i.e. she responded with abdominal movements from side to side, which is synchronized with the stimulus rotation speed. The preferred e-vector orientations (PEOs) of the bees caught at the hive entrance were randomly distributed from 0 to 180 degrees. To confirm whether a bee orients to the e-vector direction that the bee experienced during the travel to her food source, we investigated the PEOs of the bees caught at the feeder. Regardless of the feeders' locations, the bees showed significantly higher preference to the e-vector orientation coincident with that seen by the bee flying back to the hive. Our results suggest that the honeybees refer e-vector information from the skylight during their foraging flight in real time to know their heading direction.

[P032] Multiple Neuropeptide Signalling Pathways Mediate Short-Term Behavioural Plasticity in Caenorhabditis elegans

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Stimuli presented with different patterns and at different times lead to short-term behavioural plasticity, which allows an organism to quickly adjust its behavioural strategies and adapt to its immediate environment. Non-associative learning, including habituation and sensitization, are recognized as different forms of behavioural plasticity that decrease or increase the subsequent response to the same or other stimuli following experience. We use a genetically tractable model organism, C. elegans, with well-established non-associative learning paradigms, to investigate the genetic and molecular underpinnings of short-term behavioural plasticity. Our results indicated that several neuropeptide-signalling pathways are involved in modulating worms' short-term behavioural plasticity of different forms. The neuropeptide PDF is required to coordinate habituation of one response component and sensitization of another component to promote escape strategy, as a result of repeated nociceptive stimulation. Sensitization and dishabituation, both increasing the response, are mediated by different genetic and molecular components. Our research demonstrated that a wide range of neuropeptides can mediate and modulate short-term behavioural plasticity to shift between different behavioural strategies.

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