Abstracts for Poster Presentations

Alphabetical

PS3.8

Motor systems, sensorimotor integration and behavior (Part 3)

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Systematic evaluation of video acquisition parameters for mouse open-field phenotyping

Open-field assays capture ethologically relevant mouse behaviors with less experimenter intervention than highly constrained paradigms, but quantitative analysis depends strongly on video acquisition conditions. Camera placement, viewing angle, and illumination can alter body-part visibility and occlusion, yet standardized guidance for these parameters remains limited in rodent pose-estimation workflows. Here, we tested how camera viewpoint and acquisition parameters influence markerless tracking and unsupervised behavioral parsing by recording the same mice simultaneously from top and bottom views during freely moving open-field sessions in a plexiglass box under white-light illumination. Videos from 12 two-month-old C57BL/6 mice (6 female, 6 male) were collected in 15-minute weekly recordings and analyzed using DeepLabCut for pose estimation (Mathis et al., 2018) and Keypoint-MoSeq for temporal segmentation of pose sequences into behavioral syllables such as grooming, rearing, and locomotor turns (Weinreb et al., 2024). We compared Keypoint-MoSeq segmentations from matched top- and bottom-view recordings after temporal alignment. Cross-correlation of syllables showed that behavioral transitions were largely synchronized across views, indicating that some transitions were independently identified from both camera angles. We then extracted syllable-to-syllable transitions and matched corresponding events across views to assess how well transition structure was preserved. This analysis revealed an asymmetric mapping where bottom-view transitions mapped more deterministically onto top-view transitions than the reverse, suggesting that the bottom view may preserve behavioral distinctions merged in the top view. Overall, camera viewpoint is not a neutral acquisition choice but an important variable in automated behavioral phenotyping, with implications for experimental design, reproducibility, and cross-study comparability.

PS3.99

Methods

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An open-source three-dimensional digital brain atlas of a migratory bird, the Eurasian blackcap

Migratory birds represent remarkable behaviour during migration, with highly advanced navigation abilities that allow them to travel thousands of kilometres biannualy between breeding and wintering grounds, often at night and without social guidance. In addition to relying on a range of natural orientation cues, these birds possess the unique ability to sense the Earth's magnetic field. This magnetic sense has made them key model organisms for studying the neural mechanisms underlying magnetic field-guided navigation, as reflected by the identification of several brain regions implicated in magnetic information

processing. Yet, despite this progress, no easily accessible, high-resolution, three-dimensional (3D) brain atlas has been available to serve as a shared anatomical reference across studies.   Here, we present the first freely available digital 3D brain atlas for the Eurasian blackcap (*Sylvia atricapilla*), a night-migratory songbird widely used in magnetoreception and navigation research. The atlas was generated from images obtained by serial-section two-photon tomography of eight male individuals, resulting in an averaged brain template with an isotropic voxel size of 25 ?m. First version of the atlas includes 44 segmented brain regions, comprising six principal brain compartments, major anatomical subdivisions common to all avian species, regions of the song system and sensory areas (visual, trigeminal, vestibular) associated with processing magnetic field information. The atlas is compatible with the BrainGlobe Atlas API, enabling seamless integration with a growing suite of computational neuroanatomy tools. This resource facilitates accurate localization and comparison of implants, tracer injections, and neuronal populations across individuals and datasets. By providing a standardized 3D coordinate framework, the atlas promotes consistency, collaboration, and data sharing within the field of avian navigation research.

PS3.38

Social behavior and neuromodulation (Part 2)

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Effects of transdermal corticosterone on hormones and partner interactions in poison frogs

Glucocorticoids (GCs) regulate stress responses and influence social behavior across taxa. In dyeing poison frogs (*Dendrobates tinctorius*), changes in GCs are associated with stress, development, and parental care. Previous work in poison frogs demonstrated GC state-matching between breeding pairs. To test the causal role of GCs in this phenomenon, we examined how transdermal corticosterone administration affects male hormone levels, untreated female partner hormone levels, and pair interactions. Male frogs received corticosterone and a vehicle control, using a counterbalanced within-subjects design. Following each treatment, males were reunited with their female partners, and pair interactions were recorded for one hour. Water-borne hormone levels were sampled from both sexes one hour before, 1 hour after, and 4.5 hours after treatment and assayed for corticosterone and testosterone. Transdermal corticosterone significantly elevated male corticosterone at 1 hour post-treatment relative to controls, with no differences at baseline or 4.5 hours. Breeding pairs exhibited baseline state-matching of corticosterone, and following treatment, female corticosterone levels tracked those of their partners. Preliminary analyses indicate that corticosterone treatment influences behavior, suggesting a potential behavioral mechanism for hormonal coordination between partners. We predict that corticosterone-treated males will exhibit reduced testosterone levels relative to controls, consistent with GC-mediated suppression of gonadal hormones, and that partner coordination may similarly extend to the hypothalamic-pituitary-gonadal axis. By establishing an effective method for manipulating GCs in *D. tinctorius*, this study provides a critical step toward understanding the causal role of these hormones in social behavior and physiological state-matching in amphibians.

PS3.20

Motor systems, sensorimotor integration and behavior (Part 3)

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Neural responses to optic flow stimuli in the oculomotor cerebellum of zebra finches

Optic flow is encoded in two retinal-recipient regions in the vertebrate midbrain. In birds, these are known as the lentiformis mesencephali (LM) and the nucleus of the basal optic root (nBOR). These midbrain nuclei send projections to both the vestibulocerebllum (VbC) and the oculomotor cerebellum (OCb). Previous research in birds has revealed differences in connectivity to the cerebellar regions that may have functional consequences for the integration of optic flow with motor planning. Connectivity to the VbC seems to be conserved: both LM and nBOR send direct mossy fibre inputs and indirect climbing fibre inputs to the VbC. In the OCb, LM and nBOR differ in the extent of mossy fibre and climbing fibre inputs. To investigate the consequences of this connectivity, we usedNeuropixels2.0 probes to record from folia VII and VIII in the OCb of zebra finches. Birds were tested in three conditions: head-fixed anesthetized, head-fixed awake, and when tethered in a flight chamber in which the birds both perched and flew. The electrophysiological recordings were analysed using Phyllum to allow for distinction among simple spikes and complex spikes in the Purkinje cells, as well as for other cell types. For both head-fixed conditions, sine wave gratings were presented at three speeds and across eight directions. Rotational and translational optic flow was presented to both eyes together. We found a number of complex spikes in the OCb that were modulated in response to all directions of optic flow presentation. In the flight chamber, birds were presented with static gratings such that all optic signals were due exclusively to self-motion. Confirmed complex spike-simple spike associations were observed during both head-fixed and flight chamber conditions with the same probe. For the regions of the OCb from which we have recorded to date, we observed modulation of complex spikes in response to visual motion, but without directional selectivity.

PS3.18

Motor systems, sensorimotor integration and behavior (Part 3)

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Neural representation of the tutor song in the songbird basal ganglia is modulated by the premotor area HVC

Zebra finches learn their song from an adult male tutor during a brief critical period, after which the vocalization crystallizes for life. Their limited repertoire of a single song with few song elements makes zebra finches an ideal model for studying the neural dynamics of song acquisition. The songbird basal ganglia nucleus-Area X is essential for vocal development, involved in motor exploration via dopamine-mediated reinforcement during singing. Vocal learning, however, involves both active imitation via motor practice and sensory acquisition (listening to a tutor). Going beyond traditional lesion studies, which cannot isolate Area X's specific contribution to sensory processing versus motor reinforcement, we investigated the sensory role of Area X by intracellularly recording its neural activity in response to tutor song playback throughout the vocal learning period. We found Area X neurons systematically alter their activity profiles in response to this sensory input, exhibiting precisely timed, subthreshold depolarizations followed by hyperpolarizations, time-locked to specific elements of the tutor song. To identify the origin of these signals, we pharmacologically manipulated HVC, an upstream premotor nucleus projecting directly to Area X. Silencing HVC attenuated the tutor song-evoked responses in Area X, while lifting local HVC inhibition with gabazine enhanced precisely timed spiking in Area X neurons. Together, these findings highlight HVC as the primary driver of auditory responses in Area X and show that local inhibitory networks within HVC dynamically govern the strength of this sensory transmission.

PS1.52

Social behavior and neuromodulation (Part 1)

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Spectral-temporal neuronal selectivity in the awake zebra finch auditory forebrain

Perceiving complex signals like birdsong requires the brain to integrate both spectral and temporal information. However, because previous research relied heavily on anesthetized subjects, the neural dynamics of this integration in awake, behaving animals remain unclear. In this study, we investigated single units that encode spectral and temporal features in the auditory forebrain of awake, freely moving zebra finches (*Taeniopygia guttata*). We recorded units from primary (Field-L) and secondary (caudomedial nidopallium- NCM, caudomedial mesopallium- CMM) auditory regions while presenting natural conspecific songs alongside song versions selectively filtered to degrade either spectral or temporal information. Using demixed Principal Component Analysis (dPCA), we disentangled neural population responses according to spectral features versus the temporal structure of ten different unfamiliar zebra finch songs. We found distinct subpopulations of neurons preferentially tuned to either spectral or temporal features, supporting a modular organization of auditory processing. Notably, neurons in the NCM exhibited strong selectivity for spectral information, responding preferentially to natural songs, while neurons in Field-L showed noticeable temporal selectivity and included a subset of neurons that responded more robustly to spectrally filtered playbacks. Further analysis identified temporally selective neurons, especially in Field-L and NCM, often located in deeper cortical layers. The dPCA encoding weights clustered into distinct neural groups, suggesting a potential "temporal manifold," indicating that Field-L consists of low-dimensional subspaces that represent temporal song features. This study reveals specialized neuron populations in the avian auditory hierarchy: NCM neurons prioritize spectral features, while Field-L utilizes low-dimensional manifolds for temporal selectivity. Together, these distinct groups enable the robust encoding of complex conspecific vocalizations.

PS2.94

Learning, memory and cognition

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Artificial neuroevolution: illuminating resilient and robust behaviour

Artificial neuroevolution provides a powerful strategy for generating neural architectures capable of solving complex tasks. Here we present a framework developed to evolve neural circuits across generations of agents to reproduce various plastic behaviours observed in insects and other animals. Evolvable parameters not only include architecture and weights, but can also specify recurrent connections and plasticity rules. Agents can also begin with predefined minimal circuits that mimic insect ancestral brain areas, which can become more complex and interconnected through artificial evolution. We demonstrate our approach across various tasks. For instance, in an ant-like foraging task, evolved agents exhibit distinct search strategies for food discovery and homing. In a wind stabilisation task, networks exploit feedback through 'efference' copies for robust signal integration. In an olfactory conditioning task, neuroevolution produces networks capable of association between odors and reward. Across these tasks, networks remain small and interpretable, providing a framework to investigate how robustness and resilience emerge in small brains operating in dynamic environments.

PS3.5

Motor systems, sensorimotor integration and behavior (Part 3)

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Neuromuscular control of the abdomen during the flight of hawkmoths

Flying insects oscillate and deflect their abdomens during flight, a movement thought to contribute to flight control and maneuverability. However, the neuromuscular mechanisms underlying abdominal movement remain unknown. Here, we focused on the abdominal elevator muscle, the dorsal longitudinal muscle in the first abdominal segment (ab1-DLM) of hawkmoths (*Agrius convolvuli*), and investigated its role and activity during flight. Electrical stimulation of the ab1-DLM elevated the abdomen, while cutting the muscle immobilized abdominal movement during tethered flight. Free-flight assays showed that hawkmoths could still perform hovering flight for foraging after the ab1-DLM was cut; however, the body pitch angle increased following the procedure. These results indicate that the ab1-DLM is responsible for abdominal movement and flight posture control. Electromyogram recordings of the ab1-DLM during tethered flight showed periodic bursts of spikes that were tightly phase-locked to the activity of the forewing DLM, the homologous wing depressor muscle in the mesothorax. These results suggest the existence of a common motor input for wing and abdominal movements, where differences in the activity thresholds of peripheral motor systems and the mechanical properties of each joint determine whether the input results in fast oscillatory wing movement or transient deflection of the abdomen.

PS3.43

Social behavior and neuromodulation (Part 2)

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Serotonergic modulation of fight-or-flight decisions in the trap-jaw ant, *Odontomachus kuroiwae*

Speed of movement is a critical trait for animal survival. In response to unexpected threats, animals exhibit either aggressive defense or escape behavior. Hunting also requires such rapid movement. We focused on the defensive behavior of ants in the genus *Odontomachus*, which possess powerful, elongated mandibles known as trap-jaws. One of the most intriguing traits of these ants is the dual use of their mandibles for both hunting and escape. While they capture prey by snapping their mandibles at ultra-high speeds, they also exhibit either a "dart escape" or a "defensive turn"in response to tactile stimulation of the abdomen. In our observations, approximately 90% of workers responded with a dart escape, while less than 10% showed a defensive turn. The aggressiveness of ants is modulated by neurochemicals such as biogenic amines. In insects, serotonin (5HT), dopamine (DA), and octopamine (OA) are known to regulate agonistic behavior. Our measurements demonstrated that 5HT, DA, and OA levels were higher in ants that responded with an aggressive turn than in those that performed a dart escape. Oral administration of pharmacological agents revealed that a serotonin precursor(5-HTP) enhanced aggressiveness, whereas its antagonist suppressed it. This indicates that 5HT is a key neuromodulator controlling the decision to fight or flee. Furthermore, histochemical experiments identified the distribution of 5HTergic neurons in the brain and thoracic ganglia. The distribution of serotonergic terminals overlapped with the projections of mandibular mechanosensory neurons within the suboesophageal ganglion, where they directly connect to adductor motor neurons. Therefore, 5HT may regulate the sensory-motor system that controls mandible movement. This study demonstrates that serotonin serves as a key neuromodulator enabling ants to switch between fighting and fleeing in response to potential threats.

PS2.49

Auditory systems and acoustic signaling (Part 2)

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Foraging behavior of frugivorous bats using multisensory information

Animals live in an environment cluttered with signals from multiple modalities. To obtain a full image of their surroundings and make adaptive decisions, these multisensory inputs must be constantly integrated and evaluated by the brain. The frugivorous bat *Carollia perspicillata* is a specialist in multimodal integration. Firstly, these animals heavily rely on acoustic information, not only sensing their environment through active echolocation, but also perceiving a multitude of social vocalizations from conspecifics. Secondly, they depend on olfactory cues to locate food sources such as fruiting trees. But how acoustic and olfactory information is integrated during a complex behavior such as foraging, and how this information is used to make adaptive decisions is not understood. To address these questions, we investigated foraging behavior of wild *Carollia* in the tropical rainforest of Panama, when they were exposed to different olfactory and acoustic stimuli. We set up feeding stations close to several roosting sites. In pilot experiments, we presented odorants from ripe fruit (esters), fermenting fruit (short-chain acids), and plant secondary metabolites with repellent properties. Additionally, we presented playbacks of conspecific vocalizations (sequences of echolocation calls or distress calls) interleaved with silent periods from an ultrasound speaker. Approaches and feeding behavior of bats was monitored with highspeed infrared cameras and ultrasound microphones. Preliminary results reveal that bats approach feeding stations in groups of several individuals and both olfactory cues and acoustic playbacks influence foraging behavior in a stimulus-specific manner.

PS3.27

Social behavior and neuromodulation (Part 2)

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Non-nestmate discrimination in the clonal raider ant

Social insects discriminate between foreigners and members of their own colony via complex olfactory cues. How plastic this discriminatory behavior is, and whether and under what circumstances ants can learn to accept genetically distinct individuals as nestmates, is poorly understood. Here, we study this question in the clonal raider ant, *Ooceraea biroi*, which provides unparalleled experimental control over the genotype of individuals and the genotypic composition of colonies. Using a cross-fostering design with mixed-genotype colonies of wild-type and transgenically labelled individuals, we show that ants become non-aggressive specifically toward their foster genotype. This tolerance is transient, and aggression resumes after two weeks of being isolated from the foster colony. However, even sporadic re-exposure to individuals from the foster colony is sufficient to maintain tolerance for over a month, while the same paradigm fails to establish tolerance in the first place. This shows that non-nestmate discrimination is remarkably plastic and that, once established, tolerance toward foreigners can be maintained by only intermittent contact. These dynamics echo general principles of social learning and contact-dependent tolerance described in other social species, including humans. This study, in concert with recently established neurogenetic tools for the clonal raider ant, moreover opens the door to investigate the neurobiological basis of non-nestmate discrimination and the associated behavioral plasticity in social insects at the circuit level.

PS3.54

Vision and photoreception (Part 2)

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Vision in the deep: single-cell view of retinal evolution in cichlids

Sensory systems play a central role in ecological adaptation, yet the cellular and molecular mechanisms underlying visual diversification remain poorly understood in most animals.

Environments with strong light gradients provide an exceptional natural laboratory to study sensory evolution. Here, we investigate visual adaptation in cichlids from the crater lake Barombi Mbo by integrating single-cell transcriptomics, comparative genomics, and anatomical mapping to connect molecular changes with retinal structure. We generated cell-resolved retinal atlases for the shallow-water species *Pungu maclareni* and thedeep-water species *Myaka myaka*, revealing substantial divergence in cone photoreceptor composition and opsin regulation. *P. maclareni* exhibited three canonical cone classes expressing SWS, RH2, and LWS opsins, consistent with a shallow-water retina tuned for broad spectral sensitivity. In contrast, *M. myaka* retained SWS cones but lacked a distinct LWS population, relying predominantly on RH2 cones expressing RH2A?/? paralogs. These RH2 cones, which are tuned to green-shifted light, showed mixed transcriptional signatures of both RH2 and LWS identity, suggesting regulatory rewiring of photoreceptors during deep-water adaptation. In addition, fluorescent in situ hybridization revealed divergent RH2A paralog expression across ventral and dorsal retina. Furthermore, topographic maps of photoreceptors and retinal ganglion cells across multiple shallow- and deep-water Barombi species revealed shifts in acuity centers and ventral-temporal specializations associated with depth and trophic niche. Together, these results demonstrate that rapid sensory adaptation can proceed through coordinated changes in gene regulation, photoreceptor identity, and retinal organization, highlighting alternative evolutionary trajectories for visual specialization under contrasting light environments.

PS3.4

Motor systems, sensorimotor integration and behavior (Part 3)

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Neural mechanisms shape vocal divergence in *Xenopus*

Behavioral evolution arises from divergence in neural and muscular mechanisms that generate motor patterns. Vocalization is a stereotyped motor behavior essential for successful courtship across species. The *Xenopus* phylogeny comprises ~30 described species, each with a unique male advertisement call used to attract female mates. Two closely related species, *X. laevis* and *X. petersii*, diverged ~8.5 mya and both produce advertisement calls composed of fast and slow sound pulse trills. While call rates are similar between species, call duration and period differ significantly. What neural mechanisms underlie these distinct vocal patterns? We previously identified premotor neurons within the vocal central pattern generator of the parabrachial nucleus that autonomously encode species-specific call duration and period. To identify the neural mechanisms underlying vocal divergence, we are investigating the vocal hindbrain circuit in *X. laevis-X. petersii* F2 hybrids. In a fictively calling preparation, in which compound action potential (CAP) patterns resemble advertisement call pulses, we use extracellular local field potential recordings to study the premotor pattern generating circuit. Our results will distinguish whether call duration and period are regulated by shared or distinct mechanisms across parental species, providing insight into the neural basis of behavioral evolution.

PS3.3

Motor systems, sensorimotor integration and behavior (Part 3)

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Neural recordings and camouflage behavior in the dwarf cuttlefish *Ascarosepion bandense*

Cephalopods generate camouflage patterns by coordinating the action of hundreds of thousands of neuromuscular organs in the skin called chromatophores. Camouflage is visually driven: The optic lobes project to the lateral basal lobes, which in turn project to the chromatophore lobes, which contain the motoneurons that innervate the chromatophores in the skin. We are interested in how camouflage patterns are encoded in the brain and differentially represented across these levels of processing from sensory input to motor output. To parse the relationship between skin behaviors and neural activity, we are developing paradigms to evoke skin change behaviors in awake head-fixed dwarf cuttlefish *Ascarosepion bandense* and simultaneously record neural activity with calcium imaging and high-density microelectrode arrays. To identify the latent dimensions of skin behaviors, we are developing methods to decode skin patterns from neural activity and identify latent structure in the neural ensemble. These experiments should reveal the relationship between a set of skin behaviors and brain activity, to understand how the cephalopod brain implements fine motor control for skin patterning.

PS2.38

Vision and photoreception (Part 1)

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Investigating stable contrast processing across visual pathways and *Drosophila* species

For vision to work reliably in different environments, animals must encode visual features independently of viewing conditions. For instance, visual systems achieve stable, luminance-invariant contrast computation by implementation of luminance gain control. In *D. melanogaster*, stable contrast estimation under dynamic light conditions is ensured by a post-photoreceptor gain control, leading to robust behavioral responses to motion stimuli. Visual pathways implementing this gain control are known in *D. melanogaster* (Ketkar et al. 2020, 2022, 2023; Gür et al. 2024). We asked if rapid luminance gain control is a general property across states and species, and how early visual pathways are adapted to different visuoecological niches. We first aimed to test how widespread robust luminance-invariant behavior is implemented across different conditions, in different *Drosophila* strains and species. We will show that luminance-invariant behavior generalizes across *Drosophila* strains and species living in different habitats and is maintained during aging and upon starvation in *D. melanogaster*. Furthermore, not only the optomotor behavior, but also behavior relying on other visual pathways is luminance-invariant in *D. melanogaster*. However, there are species-specific differences in motion responses under bright light conditions, suggesting a species-dependent range of rapid luminance gain control. To understand where these differences are originating at the neural circuit level, we are currently establishing transgenic models for cell-type specific, or panneuronal but sparse physiological recordings in different *Drosophila* species. Our work will help us understand how the neuronal mechanisms underlying stable visual processing are shaped across species.

PS3.58

Vision and photoreception (Part 2)

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Population recordings from the lentiformis mesencephali of zebra finches to panoramic optic flow.

The processing of visual motion and optic flow is essential for effective interaction with the environment. The largest of the retinal-recipient sites in birds that encode optic flow is called the nucleus lentiformis mesencephali (LM). LM neurons possess large receptive fields and exhibit selectivity for the direction and speed of wide-field visual motion as well as in the spatiotemporal domain. An unresolved question is whether there isa topographical organization in LM with respect to receptive field location and selectivity of direction, speed, or in the spatiotemporal domain. High-density silicon probes combined with contiguous motion noise (CMN) stimuli now permit high-throughput recordings and analysis. CMN stimuli were originally developed for recordings with calcium imaging in larval zebra fish. We adapted this approach for use with much larger adult zebra finches in combination with neural spike data (spike-triggered averaging) from Neuropixels2.0 probes. As with the zebrafish, we use a globe projection to permit 360-degree visual stimulus presentation. An additional component of method development was the creation of lightweight 3D printed implant cartridges to permit reuse of Neuropixels probes for head-fixed awake recordings.

PS1.77

Evolution and development

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To court or not to court: the genetic basis of neural circuit evolution for *Drosophila* mate preferences

Behaviors that arbitrate interspecies courtship are at the crux of evolutionary barriers between species. Species- and sex-specific pheromone profiles in *Drosophila* have rapidly diversified that signify species' identity to encourage courtship among conspecifics and discourage interspecies courtship. The dominant pheromone produced by *Drosophila melanogaster* females is an aphrodisiac to their males, but the same chemical cue inhibits courtship in male *D. simulans*, whose females do not produce that pheromone. We identify a gene that has evolved to reshape the neural circuit that processes this pheromone and change the male's response from attraction to repulsion. We assess how this gene changes the anatomical and physiological properties of specific neurons in the circuit between the species to produce divergent behaviors. Additionally, we examine intra-species variation in genetic and environmental factors that alter female production of and male attraction to the pheromone cue. Together, this provides a unique mechanistic understanding of the genetics underlying neural circuit evolution and how the corresponding behavior affects evolutionary processes in the wild across different timescales.

PS2.58

Auditory systems and acoustic signaling (Part 2)

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Resolving the paradox of active sensing swarms

Active sensing animals like echolocating bats emit loud ultrasonic calls and listen for faint returning echoes to sense their surroundings. As solitary echolocators they process the multitude of the complex auditory scene comprising echoes from all the reflectors around them. Even adding one more echolocator in the same volume can already increase the amount of potentially irrelevant sensory information. The calls of another bat will mask returning echoes every now and then, while the 'non-self' echoes can cause potential energetic and informational masking. Despite these potential issues caused by conspecifics, echolocating bats remain one of the most gregarious species. Bats live in groups of hundreds to thousands while flying together in pitch-black caves. I will discuss the results of my group's work in dissecting the paradoxical proposition of active sensing swarms. How do agents compensate for the extremely dense auditory scenes in groups, and what motor strategies do they need to manage in these difficult sensory scenes. We tackle these questions using a multi-pronged approach. Our low-cost SonoRo and RoBAT swarm robotics platforms reveal the minimal sensorimotor controllers that generate acoustically mediated collective behaviour - despite a coarse angular resolution and simple sound intensity based aggregation and dispersion rules. Our minimal computational model reveals that agents do not need unique calls or echoes to echolocate and move even in large groups. Instead, agents only need to respond to sounds that arrive consistently in delay and direction-of-arrival. Our model results point to a basic, scalable sensory algorithm that filters out the sensory noise in groups. Finally, our multi-sensor data of free-flying echolocators in the field provide high spatio-temporal resolution of individual call and flight decisions in groups. The insights from our investigations are applicable to other echolocators that face similar physical and sensory constraints (e.g. avian echolocators and cetaceans). The multi-pronged approach to investigating active sensing swarms reveals the underlying behavioural heuristics while also hinting at the neural mechanisms in action.
References: Doimo et al. 2026, ANTS 2026 Beleyur & Goerlitz 2019, PNAS lab website: www.activesensingcollectives.com

PS3.41

Social behavior and neuromodulation (Part 2)

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Testing the social decision-making network hypothesis in sticklebacks with ‘omics

The neural and molecular basis of social behavior has captivated neuroethologists for decades. New ‘omics-enabled technologies, such as single-cell and spatial transcriptomics, provide an opportunity to identify parts of the brain, cell types, circuits, and molecular mechanisms important for social behavior. Here, we apply single-nucleus and spatial transcriptomics to characterize social behavior at the molecular level in threespine stickleback fish, an organism famous for its social behavior and penchant for rapid, adaptive evolution. We test the hypothesis that there is differential activity of a network of brain regions in response to social stimuli. First, we take a bottom-up, data-driven approach to identify a brain network that modulates social behavior. Specifically, we identify specific cell types associated with social behavior (parental care), determine their spatial location in the brain, and use immediate early gene expression as a marker of neural activity to estimate a functional network. We also approach the question from a different perspective by starting with a priori defined candidate genes and anatomically defined, deeply conserved brain regions hypothesized to be important for social behavior. We characterize differences in neural activity and the expression of sex steroid receptors, nonapeptides, and receptors, and TH-expressing (dopaminergic) cells and estimate functional connectivity among candidate brain regions. Overall, we find mixed support for the social decision-making network hypothesis. Our results prompt new hypotheses and questions about how deeply conserved brain regions and neurochemicals are repurposed during evolution to generate diversity in social behavior.

PS3.46

Vision and photoreception (Part 2)

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The sensory blues of *Morpho* butterflies

Morphos are very large butterflies that inhabit the rain forests of Central and South America. Most species are famous for their blue wing coloration and vividly react to blue objects. However, their wing color varies widely (from blue to brown and white) among sympatric species with distinct circadian activity, inhabiting different forest strata. This raises questions of whether and how their visual systems facilitate species-specific communication across ecological niches. To investigate how ecological factors shape signal perception, we studied nine *Morpho* species coexisting in French Guiana that differ in flight height (canopy vs. understory) and circadian activity. Using a custom insect ophthalmoscope, intracellular and extracellular electrophysiology, immunohistochemistry and anatomical analyses, we examined their retinal specializations. All studied butterflies have very large compound eyes which have a high sensitivity and acuity, allowing for long distance detection of mates and competitors. The UV-, blue-, green- and red-sensitive photoreceptors support multispectral color vision and form retinal mosaics with at least six ommatidial types. Similar mosaics are found across many other nymphalid subfamilies and are based upon three visual pigments - UV, blue (B) and long-wavelength (LW) opsins. However, Morphos have triplicated LW opsins, two of which are either blue- or red-shifted. The blue-shifted LW opsin and a blue opsin together form two receptor classes that support a dense sampling of the blue-green spectrum. The red-shifted LW opsin, together with filtering pigments, is the basis for photoreceptors with low short-wavelength sensitivity that create neural signals decorrelated from signals from blue receptors, forming ideal colour-opponent pairs. Furthermore, some species have UV and blue photoreceptors with very high polarization sensitivity. These feed into high-order neurons that function as target detectors, sensitive to blue moving objects and reacting differently to polarized and colored stimuli. While built on a generalist scheme, typical for all butterflies from the family

Nymphalidae, the eyes of Morphos contain numerous small evolutionary optimizations for viewing conspecifics - but no single silver bullet to maximally enjoy the blues.

PS2.71

Spatial orientation and navigation (Part 2)

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Projectomic reconstruction of the central complex in the fiddler crab *Afruca tangeri*

The decapod crustacean *Afruca tangeri* is a semiterrestrial crab that anchors its behaviours to a burrow. For example, during low tide, *Afruca* engage in feeding excursions throughout the tidal zone where they maintain their horizontal body axis according to the direction of their burrow, even when its location is not visible. Given they have a relatively small brain, structurally similar to other arthropods, and that their exoskeleton facilitates the implementation of in vivo chronic neuronal recording devices, fiddler crabs offer a unique opportunity to investigate how external sensory information and idiothetic cues are integrated to guide spatial behaviour. Here we aim to characterise the detailed anatomical organisation of the fiddler crab central complex (CX), a conserved brain structure implicated in spatial representation and navigation. We developed a sample preparation protocol enabling large en bloc electron microscopy to obtain a volumetric dataset encompassing the central complex, including columnar and other intrinsic neurons. Volume reconstruction reveals a structural organisation containing subdivisions of distinct neuropils, and a columnar and layered architecture with characteristic projection motifs between compartments. Comparison with well-characterised arthropod central complexes such as those of *Drosophila* and the honey bee suggests a conserved organisational logic, while also highlighting species-specific features of neuron morphology and connectivity. The resulting anatomical framework constrains hypotheses about navigation-based computations and establishes a foundation for in vivo recordings and behavioural manipulations in relatively simple, freely roaming animals.

PS1.43

Social behavior and neuromodulation (Part 1)

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Neurogenomic impact and transcriptional synchrony within distinct social relationships in the zebra finch

Forming and maintaining social bonds is critical for many species. The most well studied types of social bonds are monogamous pair bonds, which clearly support reproduction. However, many species form strong attachments with conspecifics outside of a reproductive context. Zebra finches are a powerful study system to examine the

neurobiology of social bonding, because they form strong attachments with both their opposite-sex monogamous mate and other flock members. In this study, we housed male and female zebra finches for either 24 hours or 2 weeks of co-housing with a same- or opposite-sex partner. We asked whether neurogenomic state in the auditory cortex (AUD) or nucleus accumbens (NAc) differed depending on relationship type and examined evidence for inter-partner transcriptional synchrony. We found that gene expression profiles showed more differentially expressed genes (DEGs) in male AUD compared to female AUD or NAc in either sex and four times more DEGs at 2 weeks relative to 24 hours in male AUD, most being down-regulated at 2 weeks. This may be consistent with transient shifts in gene expression during early stages of pair bonding in opposite sex pairs. In addition, we found that both AUD and NAc show extensive condition-dependent restructuring of co-expression modules as a function of relationship type and time co-housed. We then explored evidence for transcriptional synchrony between same and opposite sex partners. We found greater transcriptome-wide similarity in NAc gene expression between partners than non-partners regardless of relationship type or time co-housed. This was not observed in AUD. Finally, we tested whether individual co-expression modules demonstrate synchrony by comparing eigengene distances between partners versus non-partners. In the NAc, multiple co-expression modules showed significant synchrony with distinct modules exhibiting patterns of synchrony sensitive to relationship type and cohabitation duration. No modules in the auditory cortex showed evidence of synchrony. Synchrony in NAc may suggest that partners in either type of relationship process social reward or motivation in similar ways. Combined, this dataset offers new insights into the role of NAc in social bonding beyond monogamous partnerships and the role of sensory circuits in shaping social bond formation.

PS2.55

Auditory systems and acoustic signaling (Part 2)

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Syllable repetition reveals links between distant phrases in Bengalese finch song

Repeated execution of individual behavioral units is a common feature of many learned motor behaviors such as dance, music, and birdsong. Little is known about the neuronal control of such learned motor sequences, and specifically, how the number of variable repetitions is determined. The songs of Bengalese finches (*Lonchura striata domestica*) consist of individual syllables which can repeat a variable number of times (repeat number) to form a repeat phrase. Similar to vocal sequences in other animals, Bengalese finch song syntax is typically modelled as a Markov chain, where the choice to repeat the same syllable type or switch to a different one is made stochastically at the end of each syllable, before the next syllable is produced. Here, we report that repeat numbers of adjacent and distant repeat phrases in the song can be correlated across specific pairs of linked phrases. These hidden links between distant phrases challenge existing models of song syntax and instead suggest a hierarchical organization, where a joint factor can influence repetition of multiple non-adjacent phrases in a song bout. Repeat phrases in Bengalese finches may therefore be particularly suited to disentangle the neuronal mechanisms of how Markovian and hierarchical factors together produce a complex vocal sequence.

PS3.1

Motor systems, sensorimotor integration and behavior (Part 3)

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Snapping shrimp show evidence of altered behavior after exposure to shock waves

Exposure to supersonic high amplitude pressure waves, known as shock waves, can result in blast-induced neurotrauma. Snapping shrimp (Crustacea: Decapoda: Alpheidae) use their snapping claws to create cavitation bubbles that release shock waves upon collapse. They use these shock waves as weapons during interactions with predators, prey, and conspecifics. Along with the ability to create shock waves, snapping shrimp have evolved a helmet-like orbital hood that damps shock waves. Here, we asked if snapping shrimp with and without orbital hoods incur blast-induced neurotrauma after exposure to conspecific shock waves. We used a shelter seeking assay to measure how long it took animals to contact their burrow. We tested the same individuals sequentially to investigate individual variation. We first tested shrimp with unaltered orbital hoods prior to exposure to shock waves (armored control, AC). Then, we tested animals with unaltered orbital hoods exposed to shock waves (armored experimental, AE). Next, we tested snapping shrimp from which we surgically removed their orbital hoods but did not expose them to shock waves (unarmored control, UC). Finally, we tested the animals without orbital hoods that were exposed to shock waves (unarmored experimental, UE). Using a mixed linear effects model, we found that AE and UE animals take significantly longer to contact their shelter than AC animals. Individuals did not show significant variation throughout sequential testing ($p = 0.352$). Notably, AE animals took longer to contact their burrows than UC animals, suggesting that AE animals experience temporary deficits in motor function that are rapidly overcome. Based on these results, we will perform a longitudinal study to ask if there are temporary effects of shock wave exposure in the AE group and sustained long-term effects in UE animals. Then, we will identify physical evidence of neural damage in the brains of animals exposed to shock waves.

PS3.60

Vision and photoreception (Part 2)

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Sex-linked gene traffic underlies sexually dimorphic UV color vision in *Heliconius* butterflies: an update

The acquisition of novel sexually dimorphic traits poses an evolutionary puzzle: How do new traits arise and become sex-limited? Recently acquired color vision, sexually dimorphic in animals like primates and butterflies, presents a compelling model for understanding how traits become sex-biased. For example, some *Heliconius* butterflies uniquely possess UV (ultraviolet) color vision, which correlates with the expression of two differentially tuned UV-sensitive rhodopsins, UVRh1 and UVRh2. To discover how such traits become sexually dimorphic, we studied *Heliconius charithonia*, which exhibits female-specific UVRh1 expression. We demonstrate that females, but not males, discriminate different UV wavelengths. Through whole-genome shotgun sequencing and assembly of the H. charithonia genome, we discovered that UVRh1 is present on the W chromosome, making it obligately female-specific. By knocking out UVRh1, we show that UVRh1 protein expression is absent in mutant female eye tissue, as in wild-type male eyes. A PCR survey of UVRh1 sex-linkage across the genus shows that species with female-specific UVRh1 expression lack UVRh1 gDNA in males. Thus, acquisition of sex linkage is sufficient to

achieve female-specific expression of UVRh1, though this does not preclude other mechanisms, like cis-regulatory evolution from also contributing. Moreover, both this event, and mutations leading to differential UV opsin sensitivity, occurred early in the history of *Heliconius*. These results suggest a path for acquiring sexual dimorphism distinct from existing mechanistic models. We propose a model where gene traffic to heterosomes (the W or the Y) genetically partitions a trait by sex before a phenotype shifts (spectral tuning of UV sensitivity). Recently published publicly-available genomic data for *Heliconius aoede* provides new evidence in support of this partition-first model.

PS3.85

Olfaction, taste and chemical sensing

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Physiological bases of wound detection by the New World screwworm fly

The New World screwworm fly, *Cochliomyia hominivorax*, is an obligate ectoparasite that detects wounds in warm-blooded animals for oviposition. Upon eclosion, larvae consume the host's living tissue, producing myiasis. This fly severely impacts human health and animal welfare in South America, causing enormous economic losses in the livestock sector. Although some chemical attractants are known, the sensory mechanisms by which females locate wounds and select oviposition sites remain largely unresolved. We hypothesize a distance-dependent, multisensory integration strategy: at long distances (meters), volatile odorants act as primary navigational cues, whereas at close range (centimeters), temperature and humidity supersede olfaction to guide micro-habitat selection. To understand this host-seeking behavior, we are using behavioral assays and physiology. To identify odorants utilized for long-distance localization, we are employing Gas Chromatography-Mass Spectrometry (GC-MS) to characterize volatiles emitted by ovine feet with and without footrot (a commonly infested wound). Putative attractants will then be evaluated via olfactometry with mated females. To dissect close-range behaviors, we have developed targeted assays to isolate the roles of thermotaxis and hygrotaxis. Physiologically, electroantennograms and single-sensillum extracellular recordings will be used to map the activation of peripheral sensory neurons. Finally, we are adapting an in vivo calcium imaging technique, broadly used in model organisms like honey bees, to its use in the screwworm fly. This will allow us to record, for the first time, odorant-evoked spatio-temporal response patterns in the antennal lobe, revealing how complex natural mixtures are encoded in the brain of this devastating pest.

PS3.51

Vision and photoreception (Part 2)

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Light matters: infrared wavelengths differently affect photoreceptor health in *Drosophila*

Light influences many aspects of our well-being and the behavior of animals. All organisms evolved under the sun's spectrum, which is rich in both visible and invisible wavelengths, yet many humans and most laboratory organisms spend much of their time in artificial indoor environments. Recently, evidence has accumulated that some nonvisible wavelengths modulate specific aspects of physiology. These wavelengths often are not transmitted through windows and are absent from most electric lighting. This includes the more than 50% of the solar IR spectrum, which has been shown to positively affect aspects of physiology. This includes promoting healing of damaged tissue, leading industry to develop a plethora of red and infrared devices, the effectiveness of which often is unclear. This uncertainty largely reflects limited knowledge about which wavelengths and intensities affect physiology, although emerging evidence suggests strong effects on metabolic processes. To address this, we first induced photoreceptor damage in the eyes of white mutant *D. melanogaster*. We then monitored retinal health following exposure to the following wavelengths (in part at different intensities): 670, 750, 810, 850, and 950 nm. Assessments included electroretinograms, ultrastructural imaging, and quantification of ATP. We found that all treatments except 950 nm light facilitated improved photoreceptor function, though efficacy varied across treatments, with 850 nm light being particularly effective. Consistent with the hypothesis that enhanced recovery relates to metabolism, we found significantly increased levels of ATP. Our study represents a much needed direct comparison of the effect of multiple wavelengths and intensities and contributes to an emerging body of literature that highlights the importance of the infrared spectrum, raising questions about how to optimize both human light environments and housing of laboratory animals, including those for neuroethological research.

PS1.13

Motor systems, sensorimotor integration and behavior (Part 1)

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Interplay between syllable duration and pitch during whistle matching in wild nightingales

During complex vocal interactions, different features of acoustic stimuli are integrated to produce appropriate vocal responses, such as copying sounds during vocal matching behavior in some animals. However, little is known about the interplay and possible trade-offs between the different temporal and spectral acoustic features during these vocal exchanges. Nightingales can flexibly match the pitch of their tonal 'whistle songs' in real time during counter-singing duels. Here, we show that the syllable duration of whistle playbacks could alter the song responses of wild nightingales, causing their whistle duration distribution to shift toward the presented stimulus duration. When exposed to whistle playbacks featuring unnatural combinations of pitch and duration, nightingales demonstrate a flexible trade-off between pitch matching and temporal imitation, yet they are constrained by their vocal repertoire. They selectively adapted their vocal responses to approximate these novel stimuli, aligning them with their natural whistle repertoire. We developed a computational model of nightingale whistle-matching behavior that revealed a

hierarchical organization of acoustic feature production. During whistle matching, the feature integration process is constrained by the duration of syllables, and pitch matching follows within this temporal framework, forcing a trade-off between the two features. Our findings reveal a complex interplay between the spectral and temporal domains that shapes song-matching behavior.

PS3.32

Social behavior and neuromodulation (Part 2)

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Functional labeling of brain regions implicated in *Danionella dracula* agonistic behavior

Agonistic behavior, particularly during territorial contests, is exhibited widely among both invertebrates and vertebrates. For vertebrates, forebrain and midbrain mechanisms driving dynamic behavior during these interactions are much better understood in rodents as compared to other lineages, including fishes. Consequently, it is unclear how widely conserved agonistic mechanisms might be across the different vertebrate lineages. *Danionella dracula* (aka 'Dracula fish'), are a miniature species of teleost fish that remain transparent across life history stages from larvae to adults. Their small size and lifetime transparency make them especially amenable to brain-wide neuroimaging, thus providing a rich opportunity to study the neural circuitry of aggression in a non-mammalian adult. Male, but not female, Dracula fish are sonic and produce variable sequences of pulsatile sounds during agonistic encounters, which are often together with a striking extension and lowering of their hypertrophied, fanged lower jaw. We use a deep learning-based tracking algorithm to quantify kinematics and pose in aggressing males, examining relationships between instances of sound production, jaw lowering and other actions, including lunges and chases, over the course of a dyadic male-male resident-intruder (RI) assay. We next perform functional labeling of each individual's brain using the neuronal activity marker pERK (phosphorylated extracellular signal-related kinase). By measuring pERK levels and relating them to the frequency of behavioral actions performed during RI encounters, we identify neuronal populations associated with aggression in this species. Contextualizing these findings with proposed teleost-rodent brain homologies, we can begin to establish *Danionella* as a model to guide brain-wide optical and genetic studies of neural circuits underlying adult vertebrate aggression. Research support from NSF 2443461 and Cornell University.

PS3.31

Social behavior and neuromodulation (Part 2)

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Mapping socially responsive neuronal ensembles at single-cell resolution in bumble bees

Social behavior arises from the coordinated activation of neuronal populations that integrate and respond to social cues. Yet how insect brains encode social information at the cellular and molecular level remains poorly understood. We integrated single-nucleus and bulk RNA sequencing with HCR-FISH to characterize the cell types and brain regions

active during social interaction in the highly social bumble bee, *Bombus impatiens*. We mapped socially responsive neuronal ensembles by tracking expression levels of activity-regulated genes (ARGs), such as immediate early genes, that serve as reliable molecular markers of recent neural activation in response to social stimulation. Our snRNA-seq analyses reveal distinct gene expression patterns in neuronal clusters between bees exposed to a social partner and control individuals that experienced no social contact, including genes previously identified as ARGs in *Drosophila* as well as novel ARG candidates. Ongoing time-course expression assays and spatial transcriptomics experiments will be used to validate the transient induction of these candidates following social interaction in specific brain regions. Taken together, this work provides an early, fine-scale characterization of the neuronal ensembles responsive to social interactions in a highly social insect and advances our understanding of how social information is encoded at the cellular level.

PS1.88

Evolution and development

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3D EM reconstructions reveal diverse cell types, structural plasticity, and identification of an olfactory glomerulus in the rhinophore ganglion of a nudibranch mollusc

Gastropod molluscs have long served as subjects of neuroethological studies due to their large, identifiable neurons that are amenable to electrophysiological recording. Though considerable effort has gone toward mapping specific stereotyped circuits in gastropods, broader questions about general circuit motifs and structural plasticity have not been thoroughly explored. Although neurogenesis is known to continue across the lifespan, the abundance, location, and origin of newborn neurons in the adult ganglia have not been described. Finer-grained questions about cellular composition, neuroanatomy, and circuit organization beyond canonical circuits also remain largely unaddressed. This gap is especially apparent in the rhinophore ganglion, which is located bilaterally beneath the chemosensory rhinophore organs and thought to be a site of olfactory processing; this region primarily consists of small, unidentified neurons and has therefore been overlooked in most investigations of gastropod nervous systems. Here we used a segmented volumetric EM dataset spanning the entire rhinophore ganglion of the nudibranch *Berghia stephanieae* to characterize its cellular composition and circuit organization. At the circuit level, we found examples of rhinophore afferents converging onto projection neurons in the rhinophore ganglion, suggestive of glomerulus-like organization reminiscent of olfactory systems in distantly related taxa. We also observed many putative immature cells with growth cone-like terminations concentrated near the center of the ganglion and frequently associated with one type of glia. Our dataset contains 8221 neurons, from which we could define categories based on polarity and the direction of their axonal projections. Further, we identified and quantified four glial cell types (806 cells) with distinct morphological and ultrastructural features. Together, these findings contribute to a multilevel understanding of the gastropod brain, spanning ultrastructural, developmental, and circuit-level scales, and

reveal organizational and compositional features of an understudied molluscan sensory ganglion.

PS1.20

Motor systems, sensorimotor integration and behavior (Part 1)

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Food-associated calls hold food identity information in marmoset monkeys

Marmoset monkeys are a highly vocal and social primate species, making them a valuable model for studying the foundations of vocal communication. Previous studies have shown that their vocal behavior follows linguistic principles such as the Zipf's law of brevity and exhibits combinatorial properties within their calls. This knowledge further established marmosets as a highly flexible and promising model for investigating the prerequisites of human language. To better understand how marmosets encode information in their calls, we examined their vocal behavior in the context of food presentation - a condition known to elicit frequent and sequential vocalizations. In this project, we combined behavioral analysis with machine learning tools to characterize food-associated vocal sequences in marmosets. We found that, upon food presentation, marmosets produce complex vocal sequences that carry key acoustic modulations critical for encoding different types of foods, reflecting both broad food categories and individual food items. Our results suggest that marmoset vocalizations constitute a structured and information-rich signaling system capable of conveying detailed contextual information.

PS3.34

Social behavior and neuromodulation (Part 2)

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Quantifying multidimensional personality and behavioral consistency in *Sepia pharaonis*

Background & Objective: Animal personality-defined as consistent individual differences in behavior across time and contexts-is fundamental to understanding behavioral ecology. While cephalopods possess sophisticated neural systems, the multidimensional structure of their behavioral traits remains systematically unquantified. This study aims to establish a robust "Cuttlefish MBTI" profiling system for *Sepia pharaonis* to decode these complex individualities. Preliminary Foundation: Our prior investigations identified stable individual variation and, crucially, a decoupling between foraging success and physical aggressiveness. This suggests that "aggressiveness" and "boldness" function as distinct behavioral axes, necessitating a multidimensional model rather than a simple dominance hierarchy. Methods: We leveraged a standardized behavioral battery encompassing Boldness, Exploration, Activity, and Aggressiveness through repeated-measures designs, including refuge-use assays and mirror tests. To ensure quantitative rigor, Linear Mixed Models (LMM) were utilized to calculate Intraclass Correlation Coefficients (ICC) for trait repeatability. Subsequently, Principal Component Analysis (PCA) was applied to unveil the underlying correlation structure and behavioral syndromes, followed by K-means clustering to segment individuals into distinct phenotypic groups. Results and Implications: We found

significantly high ICC values to confirm that juvenile cuttlefish exhibit stable, inherent personalities. This framework helps facilitate a standardized MBTI-like profiling system, providing an intuitive yet rigorous quantitative tool to categorize cephalopod personality types and explore their profound influence on survival strategies and decision-making.

PS3.35

Social behavior and neuromodulation (Part 2)

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Mating-gated ovarian proteins regulate host-seeking in mosquito *Aedes aegypti*

The yellow fever mosquito, *Aedes aegypti*, poses a major public health challenge as a primary vector of deadly arboviruses responsible for hundreds of millions of infections annually. Female mosquitoes exhibit a days-long suppression of host-seeking behavior while eggs are developing. While the first phase relies on abdominal distension, sustained suppression depends on circulating signals from the ovary that persist until the eggs are laid. However, suppression is incomplete in unmated females, who continue seeking hosts even during egg development. We found that evolutionarily conserved protein signals transferred from males during mating are required to robustly suppress host-seeking in conjunction with secreted proteins from mature ovaries. Mature ovaries secrete signaling proteins that suppress host-seeking, and injecting ovarian homogenate successfully suppressed this behavior in both mated and unmated females, indicating that unmated females remain fully sensitive to the ovarian signals. Transcriptomic and proteomic analyses of gravid ovaries identified 31 candidate secreted proteins. We hypothesized that ovarian signals alone are insufficient to suppress host-seeking without a neuroendocrine environment established by mating. To test this, we are currently using fluorescent imaging to investigate whether follicles in mated females exhibit significantly stronger secretory activity than those in unmated females. We propose that unmated ovaries produce the suppressive signal but secrete it at subthreshold levels. Mating triggers the high-volume secretion into the hemolymph to achieve complete suppression. Understanding these reproductive signals that suppress host-seeking paves the way to weaponize them for preventing human-mosquito interactions, thus reducing the spread of mosquito-borne diseases.

PS1.80

Evolution and development

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Identifying the genetic underpinnings of stress response in blind Mexican cavefish

Animals perform a variety of behaviors in response to encountering stressful stimuli. While many of the molecules, hormones, and brain regions underlying stress responsiveness have been identified, the genetic factors contributing to the evolution of stress responsiveness are not well understood. To investigate the genetic basis of the evolution of stress responses, we utilize *Astyanax mexicanus*, the Mexican tetra. *A. mexicanus* is a small, freshwater fish with multiple, interfertile populations of riverine surface fish and over 35 populations of cavefish. In addition to many common cave-adapted traits like eye degeneration and albinism, multiple *A. mexicanus* populations have evolved a reduced response to stressors. We have quantified stress-associated behaviors in cave-surface F2 hybrids derived from multiple populations of cavefish and performed quantitative trait loci mapping. We identified the genetic architecture underlying the evolved reductions in stress response in multiple *A. mexicanus* cave populations, and found evidence that at least some of the same genetic loci underlie reductions in stress-associated behaviors in independently evolved cave populations. Further, we have begun to quantify stress response in surface fish with CRISPR-Cas9 induced mutations in candidate stress responsive genes. Together, these experiments increase our understanding of how complex traits repeatedly evolve, and provide future genetic targets for gene editing to further understand how changes in response to stress have evolved.

PS2.53

Auditory systems and acoustic signaling (Part 2)

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Sound and vibration sensitivity of two caecilian species *Ichtyophis javanicus* and *Typhlonectes natans*.

The caecilians, the limbless order of lissamphibians, live in humid tropical areas in all continents except Australia. Behavior and physiology of this group is not well-studied, partly due to their fossorial lifestyle. Like in many other fossorial tetrapods their middle ear is also modified. They are atympanate but have a movable columellar footplate and lack the operculum, probably as a secondary reduction. We have studied hearing and vibration sensitivity of five individuals of the Javan caecilian *Ichthyophis javanicus*, weight 9-20 g, and one individual of the larger aquatic *Typhlonectes natans*. The animals were anesthetized, placed on a vibration exciter and stimulated by dorso-ventral vibrations. In separate experiments they were stimulated by air-borne sound. We also measured the sound-induced vibrations using laser vibrometry. The vibration thresholds ranged from 1 mm/s2 at low frequencies (40-60 Hz) to 0.1 m/s2 at 1000 Hz in *I. javanicus*. The lowest thresholds in *T. natans* were approximately 10 dB higher. This sensitivity is very comparable to the vibration sensitivity of salamanders. However, the thresholds were relatively low, with lowest thresholds around 30 dB re 20 μPa. The lowest thresholds were found around 100 Hz and thresholds increased by 30-40 dB at 200 Hz to around 90 dB at 1 kHz. The finding of sensitive hearing (albeit in a restricted, low-frequency range) in *I. javanicus* is surprising. The thresholds are considerably lower than in earless frogs and salamanders, probably due to the movable columellar footplate. The sound sensitivity may be useful in navigation under ground and for detection of prey and predators. It warrants further studies of this interesting amphibian order.

PS1.63

Social behavior and neuromodulation (Part 1)

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Impact of the insecticide flupyradifurone on the hearing system in locusts and crickets

Insecticides are an essential part of modern agriculture, but their extensive use has also been associated with the world-wide decline of the insect fauna over the last decades. Therefore, it is crucial to better understand the impact of modern insecticides on non-target insects. Flupyradifurone is a relatively new butenolide insecticide that acts as a highly selective agonist at nicotinic acetylcholine receptors in the insect central nervous system. Here, we analysed the effect of flupyradifurone on the auditory pathway of the desert locust *Schistocerca gregaria* and the field cricket *Gryllus bimaculatus*. These two orthopteran insect species (*Caelifera* and *Ensifera*, respectively) have well-studied auditory networks and have evolved hearing independently. In our experiments we measured the neuronal response to acoustic stimulation while injecting increasing dosages of the insecticide into the haemolymph. In locusts we recorded the sum activity of sensory afferents in the auditory nerve and simultaneously the spike responses of the 15-20 ascending auditory interneurons in the neck connective with hook electrodes. In the cricket brain, we were able to selectively record the spike activity of the two ascending auditory interneurons AN1 and AN2 using a suction electrode. In locusts, the auditory afferents were not affected by flupyradifurone treatment, while the responses of ascending auditory interneurons decreased overall. In crickets, we found that the individual auditory interneurons AN1 and AN2 both showed increased spontaneous spiking, while the sound evoked responses decreased with increasing insecticide concentrations. Locusts and crickets as well as many other orthopteran insects rely on auditory information for intraspecific communication and predator avoidance. Therefore, the disruption of auditory processing by sub-lethal dosages of flupyradifurone may substantially impact their chances for survival and reproduction.

PS3.66

Olfaction, taste and chemical sensing

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'Virtual' odor duration and wind speeds shape *Drosophila melanogaster* search behavior

Olfactory search is an essential behavior for many animals, critical for detecting and locating food, mates, and predators. The neural and behavioral underpinnings of olfactory search are not fully known. Part of the difficulty lies in the complex olfactory stimulus-environment interactions (i.e. ambient wind) - resulting in a dynamically evolving odorant distribution in space and time. It is still unclear how duration of a recent discrete odor plume encounter affects search behavior, nor how wind ambient speeds influence the transition from the 'circling' to 'casting' search patterns described previously. We investigated the effects of altering 'virtual' olfactory stimulation duration and ambient wind speed on search behavior kinematics in freely flying *Drosophila melanogaster*. We optogenetically activated fly olfactory receptor neurons during flight, using varying stimulation durations and wind speeds, and analyzed their trajectories. Under moderate ambient wind (0.4 m/s), stimulation as short as 5-10 ms could reliably elicit upwind turns and velocity surges, although post-turn flight paths remained disorganized. Casting-like flight behavior began to emerge with 25-50 ms stimulation. Longer durations strengthened casting, increased the duration of upwind-directed flight, and increased the magnitude and duration of velocity surges. Varying ambient windspeeds resulted in trajectories resembling those seen previously, with still air eliciting 'circle-and-sink' patterned courses. Very low wind altered flight behavior, with weak upwind turns, followed by either circling or casting.

Increasing wind strengthened upwind turns and surges, while strongly biasing casting behavior, which were inhibited at the high speeds. Our results show even brief olfactory activation or under low-wind conditions, flies can compute upwind direction, make turning decisions, and orient upwind in 500 ms, suggesting neural circuitry evolved for quick, adaptive responses.

PS1.83

Evolution and development

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Inside the octopus brain: linking neural plasticity to sex-specific behaviours

Octopuses exhibit remarkable behavioural flexibility, including dynamic camouflage, observational learning, coordinated interspecific hunting, spatial navigation, and complex reproductive behaviours. Yet despite their prominence as models in neuroethology, a fundamental challenge persists: the absence of quantified neuroanatomy and a whole-brain framework capable of linking these behaviours to underlying neural architecture. We address this gap by characterising the brain morphology of the blue-lined octopus (*Hapalochlaena fasciata*) using high-resolution magnetic resonance imaging (16.4 Tesla MRI). By constructing an averaged brain template and integrating tractography with graph-theoretical approaches, we generate an annotated atlas that reveals population-level neuroanatomical variations and delineates modality-specific structures and sexually dimorphic subnetworks relevant to sensory processing and behavioural specialisation. Our analyses reveal strong dimorphism in developmental trajectories: male brains achieve maximal volume at reproductive maturity, whereas female brains exhibit slowed growth as gonads mature, followed by pronounced volumetric loss and sensory-region reorganisation during extended maternal care. Notably, chemotactile brain regions remain structurally preserved, highlighting the conservation of essential sensory pathways during physiological decline. Complementary egg-swapping experiments demonstrate that brooding females rapidly distinguish their own eggs from foreign clutches, providing behavioural evidence of robust brood recognition mechanisms. Together, these findings reveal striking structural plasticity in the octopus nervous system and illustrate how key sensory functions are maintained during the demanding, semelparous reproductive cycle. This work establishes a neuroanatomical foundation for future investigations into how octopus cognition and sex-specific behaviours emerge from dynamic, whole-brain reorganisation.

PS2.48

Auditory systems and acoustic signaling (Part 2)

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Exploring vocal production learning circuitry: methods for a novel bat model

Vocal production learning (VPL), the ability to learn to produce new vocalizations from external vocal input, is key to human language development but rare elsewhere in the animal kingdom. Among the few VPL animals, which include songbirds, parrots, pinnipeds, cetaceans and bats, bats are a particularly attractive model as they possess mammalian brain structures and are experimentally tractable. Our lab has developed the pale spear-nosed bat (*Phyllostomus discolor*) as a mammalian VPL model for exploring the neural and molecular pathways underlying this behaviour. Here, we investigate the impacts of a VPL-relevant gene on the bat brain. We developed targeted manipulations of FoxP2, a highly conserved gene known to be important for VPL. Mutations to FoxP2 cause language disorders in humans, and in songbirds, manipulations of FoxP2 revealed its dynamic role in song learning. We overexpressed FoxP2 in the *P. discolor* auditory cortex (AC), a region predominantly lacking FoxP2 in the adult bat. We then measured neural activity in response to auditory stimuli, including conspecific social vocalizations, to assess the effect of the FoxP2 overexpression. This aims to uncover how FoxP2 impacts processing of auditory information required for successful VPL-both the initial external vocal input and the auditory feedback from producing a vocalization. This research is complemented by another series of experiments aiming to map vocal motor circuitry. A major hypothesis (Kuypers-Jürgens hypothesis) predicts that direct innervation of brainstem vocal-motor neurons by higher motor brain regions is a requirement for VPL. We are testing this hypothesis by utilizing a novel system of viral tracers to trace this connection between the laryngeal muscles and the motor cortex in bats. This aims to demonstrate the presence of the direct connection in this vocal learning species, and to identify the precise region of the motor cortex that controls vocal production in bats, providing novel anatomical targets for future electrophysiology. Understanding the neurogenetic and neurophysiological underpinnings of VPL in *P. discolor* will not only further their utility as a mammalian VPL model but also provide insight into how VPL can be encoded in a mammalian brain.

PS1.74

Spatial orientation and navigation (Part 1)

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Insect-inspired olfactory navigation using an analog-constrained ring attractor and CMUT sensors

An approaching predator generates a distinct temporal sensory signature, a looming stimulus, alerting a prey. Although the mechanisms for visual looming detection have been thoroughly investigated, those associated with aerodynamic loomingó air movements induced by an approaching predatoróremain largely unexamined. Given that both types of looming involve a non-linear increase in certain signal characteristics with decreasing distance, we tested whether the mechanisms of perception of aerodynamic looming are analogous with those of visual perception. We stimulated the cercal system of the cricket, a well-established model for airflow sensing, with spider-mimicking translating spheres. The size and velocity of the looming object, the two critical parameters in visual looming perception, significantly enhanced the activity of wind-sensitive interneurons and the escape reaction. Moreover, the timing of peak firing occurred after a small delay after crossing a flow threshold, a physiological property typical of visual looming-sensitive neurons. These findings suggest the existence of convergent computational principles of looming detection across different sensory modalities.

PS2.70

Spatial orientation and navigation (Part 2)

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Steady head - steady course: how dung beetles navigate uneven terrain

Accurate orientation relies on precise steering control, which in turn requires precise representation of compass cues. Because insects' view of the sky is tied to head position and movement, extracting celestial information can be challenging for ground-dwelling insects traversing uneven terrain. How do terrain-induced disturbances affect orientation precision? What mechanisms support precise compass representation while in motion? Dung beetles provide an ideal system to address these questions. Using fine-scale head dynamics and large-scale trajectory analyses, I explore how terrain disturbances affect sensory sampling and orientation performance. Ball-rolling dung beetles rely on celestial cues to maintain straight trajectories across the savannah. During locomotion, they will experience oscillations due to the dynamics of the dung ball, as well as unpredictable body movements caused by the terrain. Both of which may degrade compass precision. Interestingly, the beetles will also intermittently stop and rotate atop their balls. Using deep learning pose estimation, I record detailed 3D movement of straight-line orienting beetles. This allows me to reconstruct head angular motion during ball rolling and dancing. Results show that the head is more stable during rotations atop the ball, an effect consistent across flat and uneven terrain. This more stable viewpoint atop the ball, combined with rotation-driven remapping of compass cues, likely increases the precision with which celestial information is sampled. By tracking long-distance trajectories with a drone, I also assess how terrain-related disturbances affect rotation frequency, path straightness, and course correction. Resulting path characteristics further support the corrective role of the beetles' rotations atop their balls. This study improves our understanding of how navigating insects cope with terrain disturbances and provides a basis for future studies on neural mechanisms supporting reliable orientation.

PS2.101

Metabolism, biological rhythms and homeostasis

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Breeding nightingales trade rest for singing

All animals share the need for reoccurring and regular sleep cycles for optimal behavioral performance, and sleep deprivation is accompanied with severe systemic consequences. Yet, some animals exhibit exceptional behavioral resilience despite apparent sleep pressure in specific etho-ecological contexts. Here, we used accelerometry and audio recordings to explore the rest/activity patterns of wild nightingales in relation to their singing performance and seasonal breeding along migratory seasons, between Europe and West Africa. Outside breeding season, they adhered to a rest/activity schedule typical for diurnal birds and dictated by the circadian light cycle, resting for ~12 hours during the night. However, breeding birds only rested for ~2 hours and spent the rest of the night singing, for several consecutive days. Further, all recorded birds in the breeding location synchronized their resting/singing activity, indicating how social and ecological pressures influence daily activity patterns. Our results highlight the adaptive flexibility of activity patterns of birds depending on different etho-ecological contexts across migratory seasons. These findings challenge the sleep-performance correlation and indicate nightingales as a novel model system to investigate sleep homeostasis in wild animals.

PS1.79

Evolution and development

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The evolution of two sensory worlds in snakes

Sensory systems are incredibly plastic over major adaptive transitions, rapidly shifting sensory perception to attune to a new sensory landscape. Evolutionary studies tend to focus on just one sensory system, however, animal senses must operate in harmony as they are linked by integrated neurological pathways in the central nervous system. Here, we leverage the ecological diversity of snakes to explore how interconnected, or resistant to change (i.e. evolvable), brains and peripheral sense organs are during adaptive transitions. We quantify volumetric changes in the neural morphology in 73 snake species using diffusible iodine contrast enhanced microCT (diceCT), which allows sensory organs, cranial nerves and brain regions to be quantified noninvasively and simultaneously across multiple ecological transitions. Surprisingly, configurations of neural traits that operate as a module are somewhat uncoupled across central and peripheral nervous system pathways. Somewhat contradicting a foundational idea in comparative neurobiology that volumetric changes should correlate according to neurological function and flow of sensory information (i.e. receptor - axon - neuron). We uncover striking convergence in senses, largely driven by the peripheral nervous system, across surface-dwelling and climbing snakes, and distinct phylomorphospace with wider disparity among burrowing and swimming snakes. Potential tradeoffs between vision and chemoreception suggest a "sensory divide" between snakes living in open air environments versus denser media of soil and water, which is representative of broader patterns across vertebrate and invertebrate lineages. Our study gives new insights into the neural mechanisms that underlie patterns of sensory investment over major ecological transitions.

PS1.32

Social behavior and neuromodulation (Part 1)

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Convergent neural expression during parental care in two neotropical frogs (Dendrobatidae and Leptodactylidae)
Parental care is more diverse among amphibians than any other tetrapod group. Amphibians exhibit 28 different modes of parental care including adaptations for attendance, guarding, transportation, feeding and protection of eggs and tadpoles. Additionally, in anurans (frogs and toads), parental care has evolved independently multiple times. To successfully perform parental care, parents undergo diverse behavioral, anatomical, physiological, and neural changes. To explore these changes at the neural level, we characterize brain gene expression from parental and non-parental individuals of two neotropical frogs from different families representing independent evolutions of care, with different parental care modes, and with differences in sex roles: *Leptodactylus podicipinus* (female parental care) and *Dendrobates tinctorius* (male parental care). Females of neotropical foam nesting frogs (*Leptodactylus podicipinus*) attend eggs in foam nests and guard free-swimming schools of tadpoles, while males of neotropical poison frog (*Dendrobates tinctorius*) care for eggs laid terrestrially and transport tadpoles to ponds upon hatching. A conserved gene expression signature associated with parental care across species will suggest independent evolutionary origins and distinct parenting behaviors are mediated by convergent mechanisms. Alternatively, distinct gene expression patterns will suggest that different modes of parental care arise via the recruitment of distinct gene sets. These are the first steps toward a larger comparative study of neotropical frogs with different parental care modes aimed at establishing general patterns of convergence versus flexibility in the repeated evolution of parental behavior.

PS2.59
Auditory systems and acoustic signaling (Part 2)
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A predictive neural code for vocalization rate in the auditory midbrain
Vocalization rate is a core temporal feature actively regulated by acoustically signaling mammals, including humans. However, the neural mechanisms by which the mammalian brain encodes this behaviorally relevant parameter remain largely unknown. By leveraging the highly dynamic vocal behavior of the echolocating bat, we demonstrate that the auditory midbrain (inferior colliculus, IC) is a critical brain region for encoding instantaneous vocalization rate. We found that most IC neurons preferentially encode vocalization rate via the reciprocal of the call interval, rather than the interval itself, a computational strategy that maximizes neural resolution the rapid temporal demands of echolocation. Surprisingly, a subset of these neurons represented both the current and future vocalization rates. Using a simple population decoding model involving only a few cells, we could decode vocalization rate with an accuracy of approximately 3 Hz. Our finding of predictive rate representations in the midbrain identifies a core neural substrate supporting the precise echolocation control in bats.

PS3.17
Motor systems, sensorimotor integration and behavior (Part 3)
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Neural circuits for visual predator recognition and evasion in *Drosophila*

Animals have evolved a variety of mechanisms to rapidly detect and evade natural enemies. In examining interactions between *Drosophila* and hymenopteran predators (parasitoid wasps), we identified two general classes of anti-predator behavior: at long range, flies orient toward wasps, whereas at close range they rapidly evade. These behaviors are vision-dependent and triggered by the sight of a conspicuous morphological cue - elongated antennae. Strikingly, antennal length serves as a threat metric: defensive responses scale quantitatively with predator antennal length and can be induced by affixing an elongated synthetic antenna to an otherwise harmless insect. Using targeted neuronal silencing, we identified distinct classes of visual projection neurons (VPNs) that mediate wasp evasion. Two-photon calcium imaging during presentation of naturalistic movies of wasps revealed complementary tuning for antennal versus body features across the identified VPN classes. Anchored on these VPNs, connectomics analysis and subsequent causal manipulations revealed two parallel visuomotor circuits - each sampling from different regions of visual space - that respectively drive orienting toward threats and escape. Together, our results reveal a simple rule for visual predator recognition based on a conspicuous predator cue, and parallel visuomotor circuits that transform the cue into context-appropriate defensive action.

PS1.76

Evolution and development

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Diet controls through JH the duration of reproduction ontogenesis in adult male moths

Many moth species are studied for the remarkable behavioural responses exhibited by male exposed to the female-released sex pheromones. By contrast, less is known about the ontogenesis of their reproduction, including the development of these responses to sex pheromones. We investigated the hypothesis that diet could modulate this development,

using male *Agrotis ipsilon* moths. In sucrose-fed male, sexual maturation occurs between the 3rd and the 5th day of adult life. Here, animals were fed various diet: sucrose alone (the reference diet, 12% w/w), a mixture of three sugars found in nectars (fructose 4%, glucose 4% and sucrose 4%) and water alone (starving condition). First, the anatomical maturation of sexual organs was assessed by dissection in 3-day and 5-day old moths. Strikingly, feeding moths with either sucrose alone or water led to similar results, i.e. sexual maturity at 5 days but not 3 days; by contrast, moths fed with the three sugars were sexually mature at 3 days. Consistent observations were made when measuring the response rate to sex pheromone in a wind tunnel; 3-day old moths fed with the three sugars behaved like 5-day old moths fed with sucrose alone. Assessing the fitness (percentage of fertilized eggs, hatching rates) led to similar results, and even to a fitness improvement at 5 days following a three-sugar diet. Overall, this indicates that a diversified diet can improve the reproductive success of male. Finally, the effects of the sucrose/glucose/fructose diet on ontogenesis disappeared when the production of JH was prevented, indicating they are mediated by this hormone. This interaction between nutrition and reproduction has important implications for the ecology of these moths, suggesting the nectars they forage on can affect their fitness. This has consequences for their biocontrol. Whether similar results can be observed in other species or in female remains to be investigated.

PS2.6

Motor systems, sensorimotor integration and behavior (Part 2)

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Divergent co-transmission by a predictive motor circuit modulates auditory processing

During behavior, an animals' movements can stimulate their own sensory afferents or shift their sensory field. For instance, whether during flight or the production of courtship song, when flies beat their wings they cause mechanosensory disturbances which activate primary auditory neurons. However, flight and courtship behavior occur under very different behavioral contexts and temporal-scales. As a consequence, the nervous system implements predictive motor circuits to modify sensory processing in anticipation of re-afferent stimulation, yet it is unclear how information about planned movements can be communicated to subnetworks that process different aspects of a single modality. Here we describe a predictive motor circuit that releases two transmitters to differentially modify auditory neurons that process sound under different behavioral contexts. In *Drosophila*, two metathoracic ascending histamine neurons (MtAHNs) become active prior to wing movement and project to the primary auditory network.

We found that the MtAHNs co-transmit the neuropeptide DH44 and histamine, and each suppress the activity of auditory neurons that are integrated into separate behavioral networks, escape and courtship, respectively. Additionally, DH44 has a greater impact on male auditory neurons and suppression by DH44 occurs over a longer time course than histamine. Disrupting either histamine synthesis or neuropeptide release by the MtAHNs also reduced courtship success with an increase in cessation during the wing extension

step. Taking a comparative approach, we found DH44 cotransmission is present in *Drosophila* species that have an auditory component to their courtship displays, but absent in species that use either purely visual courtship or lack courtship behavior. Finally, the projections of the MtAHNs into auditory centers were missing in snow flies, which are wingless. Our results indicate that divergent cotransmission by predictive motor circuits may represent an elegant means to differentially modulate sensory subnetworks impacted by a single set of movements.

PS1.41

Social behavior and neuromodulation (Part 1)

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Conserved mechanisms influence the transition to and evolutionary loss of parental care

Comparisons between behaviorally divergent populations have long been utilized in the field of neuroethology to understand the link between genes, brain, and behavior. Recent advances in species-agnostic technology, such as single-cell and spatial transcriptomics, have made powerful comparative studies possible in non-traditional model organisms. In this study, we utilize these technologies to examine two ecotypes of three-spined stickleback (*Gasterosteus aculeatus*) that have diverged in parental care. One ecotype, the ancestral 'common' ecotype, provides the species-typical male uniparental care; the other ecotype, the divergent 'white' ecotype so-called for their white breeding coloration, instead abandons their offspring by spitting embryos out of their nests immediately after fertilization. By leveraging this within-species 'natural experiment', we can gain insight into the neural basis and evolution of parental care. Single-nuclei RNA sequencing data generated from the telencephala (n=12) and diencephala (n=12) of males of both ecotypes across two reproductive stages revealed few ecotypic differences in brain cell type composition. Instead, using immediate early gene expression as a proxy for neural activity, we detected differences between the ecotypes in the activity of specific neuronal subpopulations after spawning. Whereas esr1-expressing oxytocin neurons showed increased neural activity after spawning in commons, those cells did not change in activity in whites, suggesting that estrogen-receptor mediated oxytocin neuron activity may facilitate the transition to parenthood. Additionally, differential gene expression analysis suggests both androgens and estrogens are involved in the ecotypic divergence in parental care. These results shed light on how conserved steroid mechanisms play a role in stickleback fathers' transition to, and evolutionary loss of, parental care.

PS1.53

Social behavior and neuromodulation (Part 1)

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Tettigoniid VNC: evidence for self-contained thoracic song control

Tettigoniid VNC: Evidence for Self-Contained Thoracic Song Control Rhythmic motor control in Ensifera is traditionally modeled as segmentally distributed. In field crickets (Gryllidae), the song central pattern generator (CPG) is dispersed across thoracic and abdominal ganglia [1,2]. However, our research on *Mecopoda* (Tettigoniidae) suggests a thoracic centralization of the song CPG with modular abdominal dependency for temporal precision. Using surgical lesions in the chirper-type bushcricket *Mecopoda elongata*, we analyzed ventral nerve cord (VNC) transections at the connectives between the metathoracic (T3), third abdominal (A3), and fourth abdominal (A4) ganglia. Unlike in field crickets [2], the entire song structure persists post T3-A3 lesion. T3-A3 lesions caused significant Pulse Interval slowing, while A3-A4 lesions and shams showed no robust shifts. This identifies the A3 ganglion as a source of necessary tonic drive for species-specific tempo rather than a core generator. Unilateral transection of the T3-A3 connective yielded sham-like outputs, indicating high bilateral redundancy where one intact connective maintains temporal integrity. We hypothesized abdominal recruitment would scale with call complexity. However, data shows that even for 'complex' call-type with phrased signals, the abdominal neuropile provides only stability; truncation posterior to the T3 ganglion did not disrupt the song structure. This confirms *Mecopoda* prioritizes thoracic centralization, diverging from the distributed Gryllid model. In conclusion, these findings demonstrate that the Tettigoniid song CPG is functionally centralized within the thoracic ganglia. The study identifies a hierarchical organization where the thoracic ganglia provide the core structural blueprint, while the abdominal cord acts as a modular resource for temporal stabilization. Lastly, this study also provides a functional map of the bushcricket VNC which has not been studied so far for its role underlying the control of motor behaviours. [1] Schöneich S. Prog Neurobiol 194: 101882, 2020. [2] Lin CC, Hedwig B. J Comp Physiol A 207: 533-552, 2021.

PS1.1

Motor systems, sensorimotor integration and behavior (Part 1)

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Failures that are not failures: the case of jump escape response in a grasshopper
Organisms often exhibit variability in behavior, even in response to deterministic stimuli. Such variability is commonly attributed to noise in the nervous system or unobserved internal states. The Jump escape response to a looming visual stimulus has been studied extensively in locusts to understand the behavior and the neural circuit and computation underlying the behavior. In this circuit, the Lobula Giant Movement Detector (LGMD) detects the looming stimulus and transmits information to the Descending Contralateral Movement Detector (DCMD) with near one-to-one spike correspondence. The DCMD, in turn, projects through the Ventral Nerve Cord (VNC) to Metathoracic Interneurons and Motor neurons to drive the jump response. One would expect that the jump escape response to a looming stimulus, a critical mechanism to avoid predation, should be very reliable. Yet it is observed that the locusts fail to jump in ~40% of the trials in these studies. Using the same experimental paradigm in the grasshopper Hieroglyphus banian, we observe a similar failure rate. We identify a deterministic cause underlying these apparent failures, suggesting that they reflect an alternate behavioral choice rather than random noise. Using these insights, we redesigned the experiment, and were able to rescue the behavior and significantly increase the jump escape response rate. We further perform electrophysiological recordings from the neural circuit underlying this behavior in H. banian to reveal changes in neural representations associated with jump failure as a gain modulation, and thus explain the rescue. Our findings demonstrate that behavioral

variability traditionally attributed to noise can arise from systematic, deterministic mechanisms that had remained previously unobserved.

PS1.98

Mechano- & thermoreception

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Touch-mediated egg-laying behaviour in *Aedes aegypti*

Egg-laying is a crucial developmental step in the mosquito lifecycle to ensure propagation of the following generation. As the juvenile stages are aquatic, mosquitoes must locate water bodies as sites for egg-laying. For the mosquito *Aedes aegypti*, these egg-laying sites are both artificial and natural substrates above small water bodies. *A. aegypti* uses a series of long-, medium- and short-range sensory cues to locate these small bodies of water. Once located, they will assess the egg-laying sites by relying on two contact cues, taste cues from the water body and tactile cues from the substrate. Recent work in *A. aegypti* has shown that when presented with different substrate textures, they preferentially lay eggs on rougher surfaces. We set out to build on this observation by asking three questions: 1) what specific physical characteristics of a surface induce egg-laying? 2) which sensory organs are involved in tactile sensation? and 3) what molecules mediate tactile sensation during egg-laying? We first designed a series of quantitative behavioural assays to assess how different parameters of surfaces impact *A. aegypti* egg-laying preference. Using these assays, we determined that surface depressions (or 'grooves') in the substrate above the waterline act as positive cues for egg-laying and that shallow, densely-spaced grooves are preferred. To address which sensory organs mediate this preference, we performed high-resolution videography of egg-laying females. We observed an adoption of an egg-laying posture with an elongated and curved abdomen, likely positioning mechanosensory sensilla of the abdominal tip to sense surface grooves while the animal evaluates a potential egg-laying surface. Finally, to better understand the molecular mechanisms of tactile sensation, we have used CRISPR/Cas9 to develop a knock-in/knock-out mutant in *A. aegypti* ppk322, the gene ortholog of the mechanosensitive DEG/ENaC channel ppk in *Drosophila melanogaster*. This mutant will allow us to further dissect the cellular and molecular mechanisms of touch during egg-laying and other behaviours in *A. aegypti*. Altogether we hope that these findings will shed light on future mosquito population control strategies and reduce the spread of mosquito-borne diseases.

PS1.61

Social behavior and neuromodulation (Part 1)

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Network analysis of vocal exchanges in a wild rodent

Social living requires coordination to maintain group cohesion, promote cooperation and reduce conflict. To this end, many social species use contact calls during group activities to share information. While communication is often studied in pairs of individuals, vocal interactions in natural groups typically involve multiple signalers and listeners. Network approaches can link individual interactions to group dynamics by integrating (i) social networks (who interacts with whom), (ii) vocal networks (who exchanges calls with whom), and (iii) communication networks (who can hear whom). However, these interdependencies are rarely quantified due to methodological constraints.

We investigate how these networks shape vocal exchanges in the African striped mouse (*Rhabdomys pumilio*), an emerging model for neuroethology of social behavior. This highly social murine lives in family groups up to 25 individuals who share a nest and bask together at sunrise (cooperative context). They are however solitary foragers during the day, and we observe varying degrees of tolerance and competition when several mice forage at the same patch. In both of these contexts, striped mice are highly vocal and produce ultrasonic contact calls.

We record three wild groups during basking and at food patches using an eight-microphone array for individual call assignment, combined with video-based tracking and identification. Using these data, we construct social, vocal and communication networks and examine how their structure and relationships vary across cooperative to competitive contexts.

We predict that vocal exchanges rule will vary with group dynamics, with cooperative context exhibiting more coordinated turn-taking than competitive contexts. We further examine whether disruptions of timing rules predict social events and depend on social ties. Finally, we construct communication networks to test how acoustic connectivity shapes vocal exchanges in between clusters.

PS2.78

Spatial orientation and navigation (Part 2)

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Cholinergic receptors as candidate regulators of migratory behavior in Swainson's thrushes

Understanding the molecular mechanisms that control migration remains a major challenge in behavioral genetics and neurobiology. We are leveraging the hybrid zone between coastal and inland subspecies of Swainson's thrush (*Catharus ustulatus*), which differ in migratory orientation, distance, and timing, to identify genetic and neural mechanisms underlying migratory behavior. Using tracks from hundreds of free-flying birds, we quantified multiple migratory traits and used admixture mapping to identify genomic regions associated with these traits. Functional enrichment analyses of candidate loci identified 192 genes associated with migratory traits, with significant enrichment for genes involved in nicotinic acetylcholine receptor activity. We conducted a complementary comparative transcriptomic analyses using captive birds, focusing on five brain regions associated with migration in parental and hybrid birds sampled across migratory and non-migratory seasons. These analyses identified differential expression of genes involved in circadian rhythms and neural development, including nicotinic acetylcholine receptor genes. The convergence of population genomic and transcriptomic results suggests cholinergic signaling pathways may play an important role in regulating migratory behavior. We will expand on future work designed to more directly test the role of cholinergic receptors in regulating migratory behavior in these thrushes, including spatial mapping of gene expression using in situ hybridization and experimental manipulation of receptor activity using pharmacological approaches.

PS1.58

Social behavior and neuromodulation (Part 1)

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Neuronal entrainment to ecologically relevant rhythms in the auditory cortex of bats

The auditory specialist *Carollia perspicillata*, a frugivorous bat, lives in a rich acoustic environment, comprised of a multitude of simultaneously ongoing vocalizations in the colony. An important feature of this complex soundscape is its rhythmicity, which may be exploited by the bat's auditory system to predict upcoming stimuli and guide behavior. However, the way neuronal activity in the auditory system entrains to such rhythmic stimuli is incompletely understood. We presented head-fixed, awake bats with rhythmic acoustic pulse trains in several temporal frequencies naturally present in their environment, while recording firing rates in the auditory cortex. Neurons showed clear tracking of the stimulus phase at the single-cell level, especially at 6 Hz, a rate which was also observed to be the rate at which bats most commonly repeat distress vocalizations. At the population level, neurons tracked the stimulus phase of all tested frequencies, performing better than at the single-cell level. Analysis of the spikes after the stimulus offset also gave some evidence of entrainment echoes, a key marker of neuronal entrainment. Taken together, this evidence puts the auditory cortex in a privileged position to follow and predict ecologically relevant rhythms in the bat's environment.

PS3.63

Vision and photoreception (Part 2)

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Visual function and cell-type specificity during regeneration in the axolotl

The axolotl (*Ambystoma mexicanum*) is a powerful vertebrate model for studying nervous system regeneration. While anatomical regrowth of the visual pathway is documented in other amphibians, quantitative data on the restoration of meaningful function—and specifically how precisely regenerated circuits are organized—remains largely unknown in the axolotl.

To address this, my PhD project asks whether the functional mapping between distinct retinal ganglion cell (RGC) populations and specific visual behaviors is accurately restored during regeneration. To enable this research, we established a standardized visual behavior paradigm that quantifies prey-capture responses and detects the loss and recovery of vision following optic nerve transection. Building on this foundation, my project focuses on resolving visual system regeneration at increasingly specific scales of organization.
First, I will go beyond axonal repair by establishing tools for pan-RGC labeling and inducible ablation using the nitroreductase (NTR) system. In contrast to optic nerve transection, which preserves retinal neurons while severing their axons, this approach allows me to test whether these neurons can regenerate *de novo* and restore visual function.

In a next step, I will move toward circuit specificity by targeting molecularly defined RGC subtypes. Using candidate genes identified from single-cell transcriptomics, I am developing fluorescent reporter lines to label and selectively ablate distinct RGC populations. This will first define the contribution of these subtypes to specific behaviors such as prey capture, phototaxis, and looming escape. I will then test whether these cell-type-specific behaviors are restored with different temporal dynamics or success rates during regeneration.

Together, this work aims to determine how precisely visual circuits are reassembled and whether regeneration preserves the functional logic of cell-type-specific organization in a tetrapod model.

PS1.93

Mechano- & thermoreception

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Pilifers provide proprioceptive feedback about proboscis motion during feeding in hawkmoth, *Manduca sexta*

Hawkmoths precisely guide their long and flexible mouthpart called the proboscis along the floral surface to reach the tiny nectary opening on the flower leading to nectar in the floral spur. The proboscis is continually guided by tactile and visual sensory feedback during this process as the moth hovers in front of the flower. Even after moths insert their proboscis into the nectary spur, they continue to track floral movements as the flowers sway in the wind. Hawkmoths' ability to track floral surface cues and floral motion requires feedback about relative proboscis motion. How moths sense and control proboscis motion to achieve precise movements remains an open problem. Interestingly, there is a pair of hair-like mechanosensory bundles, called pilifers, located at the base of the proboscis that could provide such proprioceptive feedback. Using extracellular multi-site recordings, we asked whether pilifer afferents are sensitive to small lateral movements of the proboscis, typical during floral exploration and tracking. We found that they respond to unilateral deflections of about 5-10 degrees either to their left or right, with a mean latency of 1.02 ± 0.48 ms (mean ± std). Different neurons are tuned to different directions, extent of motion, and speed of motion. We used reverse correlation analysis to build 1D computational neural models which allow us to predict their responses to natural stimuli. Additionally, neural tracing revealed extensive sensory arborizations into the sub-esophageal ganglion of the brain, and further into the ventral nerve cord, indicating a feedback pathway to the proboscis, neck and flight motor systems to enable rapid coordination of appendages and body for successful feeding.

PS2.92

Learning, memory and cognition

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Electrophysiological exploration of time perception in an invertebrate: the common cuttlefish (*Sepia officinalis*)
Cephalopods represent a particularly advanced source of knowledge in neuroethology. With a brain architecture that is profoundly different from that of vertebrates and unique among invertebrates, these mollusks demonstrate complex cognitive abilities. Indeed, numerous behavioral studies conducted on cuttlefish have highlighted their advanced cognitive abilities: associative learning, long-term memory, and even behavioral adaptation in anticipation of a future event. However, the brain mechanisms underlying these abilities remain largely unknown. In particular, the functional properties of this cephalopod's brain are very poorly characterized in vivo, which limits the ability to establish direct links between behavior and neurophysiology. In this regard, our study aims to adapt a method for recording brain activity in cuttlefish under wakeful conditions. Among the cognitive dimensions that can be explored using this approach, time perception represents also an area that remains unexplored in this species. The analysis of evoked responses thus serves as a tool for describing how the nervous system processes short-term temporal information. By varying the durations and frequencies of light stimuli presented as flash trains during recordings, we aim to identify the components of these responses that are specifically associated with the processing of temporal information. This initial technical and analytical development phase will subsequently enable a more in-depth study in the context of temporal discrimination.

PS2.21
Motor systems, sensorimotor integration and behavior (Part 2)
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Flies use gyroscopes to increase the precision of their internal compass
The insect order Diptera is widely acknowledged as the most ecologically diverse groups of insects1. Although two other orders are larger in terms of the number of described species, the ~160,000 species of flies are noteworthy with respect to their astonishing behavioral diversity and ability to converge upon the natural history and morphology of other insects. In contrast, the members of other insect orders are far less successful at encroaching upon the life histories at which flies excel. What are the unique specializations that may have allowed dipterans to succeed at so many different flight behaviors compared to other insect orders? In this work, we show that flies use angular velocity measurements from their halteres to increase the precision on their navigational compass, thus providing them with unique capabilities that may have catalyzed their remarkable behavioral diversification. Halteres function as gyroscopes to encode angular velocity during flight. Their likely ancestral role was to detect perturbations about the yaw, pitch, and roll axes during flight and initiate rapid compensatory reflexes via rapid local connections to steering motor neurons in the ventral nerve cord. Here, we show that the haltere sensory pathway also provides critical input to the animal's navigation system within the specialized region of the brain called the Central Complex. By exploiting the underlying physics, we implemented a method for accurately simulating angular velocity using micro-oscillation of the fly's body that is phase-locked to the haltere stroke cycle2, allowing us to perform 2-photon imaging and whole cell patch recording of individual neurons in the brain of flying animals subjected to rotations exceeding 2000 deg s-1. Because the mechanosensory-based halteres can

encode high angular velocities that are well beyond the temporal resolution of the visual system, flying flies can accurately update their compass heading during rapid turns, providing a distinct performance advantage over all other insects. We propose that this unique role of the halteres in the compass system is responsible for the extraordinary ecological diversity of flies. 1. Grimald, D & Engel, M (2005). Evolution of the Insects. Cambridge. 2. Nalbach G, and Hengstenberg, R (1994). J. Comp. Physiol. A, 175, pp.695-708.

PS1.10

Motor systems, sensorimotor integration and behavior (Part 1)

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Wild or cultivated? – differences in flower handling efficiency of insect pollinators

Humans have cultivated ornamental plants for thousands of years. They are mainly used as decorative elements and therefore often bred to possess enhanced and altered visual traits, like changes in flower colour, patterns and size. These differences in visual traits from their wildtype counterparts are based on human visual preferences and perception. However, for insect pollinators, these visual floral traits are important for all stages of flower handling, including approach flight, landing guidance and nectary search, allowing pollinators to handle flowers more efficiently. We expect pollinators to exhibit more efficient flower handling, when visiting wild types than ornamentals, because they co-evolved with wild-type plants. Although interactions between insect pollinators and ornamental plants have been studied, no detailed investigation of how their visual appearance impacts insect pollinators' flower handling exists. In this project, we aim to understand how the visual differences of wildtype and ornamental plants impact the efficiency of flower handling by insect pollinators. We use the buff-tailed bumblebee and hummingbird hawkmoth as model organisms, as both species rely heavily on visual floral traits for foraging and represent two major pollinator groups, Hymenoptera and Lepidoptera. We present these animals with real flowers for foraging in a laboratory setup. Presented flowers were chosen based on citizen science observations of pollinator visits to different plant groups. Flower visits are recorded and tracked using trained neural networks. We extract foraging time as well as trajectory characteristics. By investigating the differences in pollinators' ability to handle wildtypes and ornamentals efficiently, we gain a more detailed understanding of the coevolutionary dependency of plant-pollinator interactions in terms of visual signals and the impact of the changing floral landscape on these interactions, caused by the introduction of ornamental plants.

PS2.62

Auditory systems and acoustic signaling (Part 2)

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Bats dynamically shift harmonic energy to counter acoustic interference

Previous studies of noise-robust echolocation in bats have primarily focused on adjustments of the dominant harmonic, overlooking potential coordination among harmonics. Here, we test whether *Hipposideros pratti* flexibly regulate harmonic structure under broadband noise, narrowband noise, and heterospecific acoustic interference.

Across conditions, all harmonic amplitudes increased with increasing noise levels. However, narrowband noise induced frequency-specific modulation: when noise overlapped with the second harmonic, amplitude increase for all harmonics, whereas overlap with the first harmonic only induced increases of the first harmonic, and overlap with the third harmonic produced no significant changes. Under heterospecific acoustic interference, amplitude modulation was generally coordinated across harmonics, except for Myotis pilosus calls that elicited disproportionately greater increases in the first and third harmonics. These amplitude changes also led to corresponding increases in the power ratios of H1 and H3. Specifically, bats showed a selective increase in the power ratio of H1, but not H3, in response to acoustic interference, indicating asymmetric redistribution of acoustic energy across harmonics. Together, our results reveal a previously underappreciated mechanism in which bats maintain echolocation robustness through flexible, coordinated control of harmonic amplitude.

PS1.46

Social behavior and neuromodulation (Part 1)

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Mapping manipulation: spatial metabolomics of hairworm-infected cricket brains

Many parasites have evolved the remarkable ability to alter the phenotype of their host to facilitate transmission, a phenomenon known as host manipulation. Freshwater hairworms (Nematomorpha: Gordiida) notoriously induce their terrestrial insect hosts to enter water, where the parasite emerges and completes its reproductive cycle. Although infected hosts exhibit altered activity patterns and responses to environmental cues, the neurochemical mechanisms underlying these behavioural changes remain poorly understood. Here, we aim to investigate the spatial metabolic landscape of parasite-induced behavioural manipulation using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). Building upon our recent behavioural and multiomic studies using the cricket *Acheta domesticus* experimentally infected with *Paragordius varius*, we generated spatially resolved metabolomic profiles of the central nervous system during the critical time point when infected crickets enter water. By integrating these data with established behavioural paradigms, such as responses to long- and short-distance cues, we seek to identify neurochemical signatures associated with altered sensory processing and host-seeking behaviours that favour parasite transmission. To our knowledge, this work will provide the first spatially explicit view of metabolic changes in the nervous system of a parasite-manipulated host. By mapping molecular distributions within the cricket brain, we aim to uncover candidate pathways and regions involved in behavioural modulation, offering new insight into how parasites ‘drive’ host behaviour at the neurochemical level.

PS2.51

Auditory systems and acoustic signaling (Part 2)

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RO-BAT and SonoRo: bio-inspired swarm robotics platforms for audio-driven navigation.

Bats achieve agile flight and coordinated collective motion by relying on echolocation, even within dense swarms where numerous overlapping acoustic signals give rise to the so-called Cocktail Party Nightmare [1]. Bats can detect the location of nearby individuals in two ways: either through active echolocation by emitting calls and listening for their echoes, or by passively listening to the calls and echoes produced by their neighbours. Inspired by these remarkable capabilities, we introduce RO-BAT and SonoRo, two bio-inspired robotic platforms designed to explore principles of echolocation and acoustic sensing. SonoRo is a passive sound-localisation platform that locates external sound sources listening to external beacons. We equipped SonoRo with an array of microphones for sound localisation and a loudspeaker to broadcast beacon sounds. We were able to demonstrate SonoRo in four acoustic navigation scenarios: collision avoidance with static sound sources, goal finding in a static environment, multi-agent aggregation, and multi-agent dispersion. (Video of the SonoRo experiments: https://youtu.be/8sPeUnS8U3I) Building on the SonoRo, we then implemented active echolocation in the RO-BAT. We developed a signal processing pipeline capable of extracting both range and directional information from returning echoes. Laboratory experiments demonstrated that the RO-BAT could autonomously detect and avoid obstacles using only self-generated acoustic cues, effectively reproducing the core principles of bat echolocation in a robotic platform. Our current research focuses on scaling up swarms of autonomous SonoRos and RO-BATs into fully decentralised collectives of agents, enabling a wider repertoire of swarm behaviours inspired by echolocating swarms. As part of an interdisciplinary research programme, we pursue parallel investigations into active sensing and the development of novel robotic systems. We hypothesise that strategies evolved by bats to cope with overlapping acoustic signals can inform scalable and resource-efficient sensing in robotics.

[1] Beleyur, T. & Goerlitz, H. Modeling active sensing reveals echo detection even in large groups of bats. Proceedings of the National Academy of Sciences 116, 201821722 (2019).

PS1.54

Social behavior and neuromodulation (Part 1)

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Spatial mapping of mating-related neurons in the malaria mosquito brain using HCR

Mating in the malaria mosquito, *Anopheles gambiae*, requires males to acoustically locate females within male-dominated swarms. During these daily swarms at dusk, males deploy mating-specific auditory modulation, controlled in part by the efferent neuromodulation of the Johnston's organ. However, how the brain regulates auditory-preparedness for mating remains largely unexplored. To elucidate these underlying neural mechanisms, we are establishing a comprehensive cellular map of the *A. gambiae* brain. Utilizing our single-nuclei RNA sequencing (snRNAseq) data, we are spatially mapping neuronal populations using Hybridization Chain Reaction (HCR) RNA-FISH - a technique recently optimized for *A. gambiae*. Crucially, we are combining this with brain registration, to generate a comprehensive spatial atlas of transcriptomically identified neurons. Finally, we are studying mating-related neuronal populations by comparing brains from different behavioural states and housing conditions, and utilizing Activity Regulated Genes (ARGs) as readout of neuronal activation. Understanding this tightly controlled spatiotemporal behaviour may allow opportunities for novel control methods in the fight against malaria as well as providing a model to study how behavioural state enhances sensory perception.

PS1.89

Evolution and development

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Conserved olfactory neuron cell types and rapidly evolving receptors across the mosquito antenna

The sense of smell is a critical interface between organisms and their environment, allowing animals to detect food, mates, predators and other resources. We might therefore expect the genes and cells that underlie odor detection to evolve rapidly. Indeed, olfactory receptors are often the most rapidly evolving genes in animal genomes, blinking on and off across species as genes are duplicated, deleted, and evolve novel tuning. Much less is known about the evolution of the sensory neurons in which these receptors are expressed. For example, mosquitoes are an ancient family of flies that date back 180 million years. Their odorant receptors evolve rapidly, with fewer than 10% of genes having 1-to-1 orthologs across species, yet all species appear to have the same number of sensory neuron cell types. Are these cell types conserved or do they undergo a rapid birth-death process similar to receptors. To answer this question, we combined preexisting single-nucleus RNA sequencing data from *Aedes aegypti* antennae with newly collected data from three additional disease-vectoring mosquitoes-Aedes albopictus, *Culex quinquefasciatus*, and *Anopheles coluzzii*-totaling 328,569 nuclei at 20-50x antennal coverage. The number of cell types and proportional representation of major neuron classes-including Orco+ and Ir8a+/Ir76b+ neurons-are broadly conserved across all four species. Cross-species integration into a shared expression space further reveals that conservation extends to regulatory identity. While we can identify a few clear cases of cell type gain or loss, our data suggest that olfactory sensory neuron cell types are stable evolutionary units even as receptors turn over rapidly. Finally, we examine the evolution of receptor co-expression across cell types, placing co-expression patterns in their genomic and phylogenetic context to distinguish co-expression by co-option from co-expression by shared descent. Together, these findings suggest a surprising decoupling in the pace of olfactory evolution: rapid receptor turnover plays out against a backdrop of conserved sensory neuron architecture.

PS1.71

Spatial orientation and navigation (Part 1)

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Turtle ants reshape nest networks in response to interspecific competition

Social insects are well-known for strategically cooperating to obtain resources and defend against competitors. Most social insect colonies cohabitate a single nest, however many tree-dwelling ant species have increased their potential for resource sharing and territory defense by building networks of multiple nests per colony within the forest canopy. This

study explores how arboreal ants operate within the spatial constraints of their environment to create defensible nest networks when there is interspecific nest site competition. This question was explored with two species found in the Florida Keys: Northern Caribbean turtle ants (*Cephalotes varians*) (native) and graceful twig ants (*Pseudomyrmex gracilis*) (non-native). Colonies of these species compete within and between species for a limited number of nesting cavities on trees. To determine if competition on a single tree causes *C. varians* to modify their nest network by changing nest location and/or shifting population distribution across nests, we performed three levels of competitive interaction experiments: one-on-one encounters, pheromone exposure, and full-colony interactions. Our results showed that *P. gracilis* was consistently aggressive toward *C. varians* individuals and colonies, however *C. varians* followed the foraging paths of *P. gracilis* and survived almost all one-on-one altercations. During full colony interactions, *C. varians* colonies consistently chose to place at least one nest adjacent to a *P. gracilis* nest. *C. varians* defended its nests against attack using specialized armor and overwhelming numbers. Compared to when no competitor was present, *C. varians* created nest networks with more direct paths between nests, simplifying travel between nests. This study has revealed that *C. varians* relies on spatial decision-making abilities and morphological defenses to limit risk while traveling over territories shared with other species.

PS1.99

Mechano- & thermoreception

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Contribution of dynein motors to active hearing

Dynein motor arms are thought to contribute to active mechanosensation. In *Drosophila*, the chordotonal sensory organ, Johnston's Organ (JO), contains subsets of neurons with specialized properties for different sensory modalities. Our work focuses on the contributions of dynein motors to the vibration-sensitive (acoustic) and static deflection-sensitive (wind or gravity) subsets. Null mutations in smetana (smet), which encodes dynein axonemal assembly factor 1 (DNAAF1), result in loss of dynein arms in all JO neuronal subsets. We show that complete loss of smet function produces a deaf phenotype measured by electrophysiology, and abolishes active antennal vibrations, as measured by laser vibrometry. To distinguish the effects of dynein arm loss on distinct sensory modalities, we are using the GAL4-UAS system to manipulate smet gene expression independently in vibrational and static-deflection subsets. Electrophysiology recordings demonstrate that reduction of smet expression by RNAi in vibrational subsets results in significant hearing loss, whereas no hearing loss is observed following smet knockdown in static deflection-sensitive neurons compared with controls. These results are consistent with a model in which dynein arm motors make distinct contributions to different mechanosensory modalities. A complementary approach using modality-specific rescue of the smet mutation is currently in progress.

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PS1.90

Evolution and development

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Estimating the sensory abilities of the extinct Great Auk (*Pinguinus impennis*)

The extinction of the Great Auk (*Pinguinus impennis*) in the mid-19th century represents one of the most dramatic and well-documented losses in recorded history. While previous studies have examined aspects of its ecology, much of its behaviour remains uncertain. Comparative anatomy can provide insights into the Great Auk's sensory abilities because acuity and sensitivity are closely linked to the structure of sensory organs and brain regions that process sensory information. The internal morphology of the avian braincase closely reflects the brain, and because the skull houses both the brain and sensory organs, cranial anatomy offers a valuable proxy for reconstructing sensory capabilities in extinct species. Here, we use a quantitative approach to examine the cranial and endocranial anatomy of the Great Auk relative to 13 closely related species (puffins, murres and other members of the family Alcidae). Using measurements from micro-CT scans and endocasts, we quantified visual (orbit diameter, optic lobes surface area), tactile (area of the ophthalmic and maxillomandibular branch of the trigeminal foramen), and olfactory (olfactory bulbs volume) structures relative to brain volume, to test whether the Great Auk differed significantly from related species in any measured trait. Our analyses reveal that the Great Auk possessed visual and olfactory capabilities consistent with those of extant alcids, scaling isometrically with its brain volume. Notably, its relatively enlarged ophthalmic branch of the trigeminal nerve indicates enhanced tactile sensitivity in the upper bill, which might have supported tactile based foraging in the low light conditions characteristic of its deep diving, high Arctic niche.

PS2.31

Vision and photoreception (Part 1)

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Neural circuit tuning to visual statistics in the larval zebrafish

Visual systems encode and internally represent relevant information to guide adaptive behavior. For animals to survive and reproduce, this encoding needs to operate efficiently in highly variable natural contexts. The plethora of niches, such as muddy waters for zebrafish or a dense jungle for a capuchin monkey, hint at different challenges each visual system needs to overcome. Thus, the statistical structure of visual input is considered a driving force in shaping vision. Evolutionary constraints have led to specific hard-wired adaptations of neural circuit structure and function that capture patterns of an animal's environment. To what degree these properties can be flexibly tuned to the environment during early development and in later stages is still debated. To address this question, we employ a combination of virtual reality behavioral experiments, whole-brain two-photon imaging, and molecular analyses, using a small, transparent vertebrate - the larval zebrafish - as a model system. These experiments allow us to explore in detail how different features of visual scenes modulate behavior and shape neural structure and function. We find that both spatial and temporal statistics of the visual world during development influence the swim kinematics of fish in both static and moving environments. We link these changes to the modulation of functional responses of neurons in the pretectum and optic tectum, showing that the visual system adapts to the structure of the visual environment to guide behavior. Then, we embed these findings in a theoretical inference-based framework to

explore the general principles of vertebrate visual circuit tuning. Our results reveal the circuit mechanisms that allow a vertebrate visual system to flexibly shape behavior based on early sensory experience, enabling unprecedented dissection of the underlying functional and molecular processes in the brain.

PS3.37

Social behavior and neuromodulation (Part 2)

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Species differences in behavioral and neurotranscriptomic synchrony during tadpole fighting

Coordination plays a key role in social interactions, as animals rely on others' behavior to adjust their own actions. This coordination can reduce risks during aggressive encounters if increased behavioral synchrony facilitates assessment of relative strength and thereby termination of fights with minimal injury. However, the degree of behavioral synchronization may vary across species, reflecting differences in fighting strategies or sensitivity to social cues. Here, we compared behavioral synchronization during pairwise fights between tadpoles of two poison frog species, *Dendrobates tinctorius* and *Ranitomeya imitator*. To test whether behavioral coordination is accompanied by neural coordination, we used RNA-seq to assess gene expression synchrony in the brain. We found that both species exhibited significant movement synchronization during fights, but *D. tinctorius* showed stronger synchronization than *R. imitator*. In brain gene expression, we identified genes that were synchronized between fighting partners relative to random pairs in both species. In *D. tinctorius*, these genes were enriched for stress-response functions, whereas no such enrichment was observed in *R. imitator*. Species differences in synchronization may reflect variation in developmental environments, as *D. tinctorius* tadpoles develop in larger ponds, where higher encounter rates lead to resource competition, which may favor stronger responsiveness and synchronization during fights. Our findings suggest that adjusting aggressive behavior to achieve synchronization may be generally important; however, the degree of synchronization can vary across species, and this variation may be linked to variation in brain gene expression responses.

PS1.75

Spatial orientation and navigation (Part 1)

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Neuroethology of multi-species neural coding in the hippocampus of flying bats

The hippocampus is essential for navigation and contains place cells, neurons that encode an individual’s self-location. However, natural navigation occurs in complex environments populated by various agents, including conspecific animals, other species, and behaviorally

relevant objects. To understand how the hippocampus encodes such agents during navigation, we performed wireless neural recordings from dorsal-CA1 hippocampal neurons of wild-caught bats, as they flew toward conspecifics (other bats), heterospecifics (humans), or objects. Hippocampal neurons encoded both the agent's class (humans/bats/objects) and identity (e.g. human1/human2 or bat1/bat2). The encoding mechanism was via gain-modulation of place fields ('rate-remapping') - which allowed coding of agent class and identity while preserving spatial tuning. The neuronal population activity formed a hierarchical representation, with agent identity being nested within the representation of agent class (species). Thus, during navigation, hippocampal neurons formed a structured code of agent classes and identities in parallel to the classical spatial code.

PS3.71

Olfaction, taste and chemical sensing

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Neural basis of mosquito host-seeking

A single mosquito bite can be deadly. By biting humans, mosquitoes transmit pathogens responsible for malaria, yellow fever, Zika, and dengue, which together claim more than half a million lives each year. Only female mosquitoes bite, as they require a blood-meal for egg maturation, and they rely on the integration of sensory cues to locate human hosts. Among these cues, carbon dioxide (CO2) from human breath plays a fundamental role. CO2 detection triggers activation, transforming mosquito behavior from undirected exploratory flight to sustained targeted upwind navigation, while simultaneously initiating sensitization, in which other modalities become primed to respond strongly to host cues such as body odor, heat, and visual contrast. Together, activation and sensitization initiate the host-seeking behavioral program that culminates in mosquito biting. Host-seeking is remarkably robust: mosquitoes lacking many major chemosensory receptors still successfully locate humans. Recent anatomical advances revealed that CO2-sensitive sensory neurons possess high recurrent connectivity and ribbon-like synapses that likely amplify peripheral detection. However, the neural basis of host-seeking remains unexplored. The circuit architecture, functional connectivity, and neuronal response dynamics that transform sensory input into directed host-seeking have not been experimentally characterized. I am addressing this gap using whole-cell patch-clamp electrophysiology in adult mosquito brain preparations, combined with dye filling and registration to a standardized reference brain and a synapse-resolution connectome to anatomically identify recorded cells and map their connectivity. I am measuring physiological and synaptic properties across second-order sensory neurons to reveal how olfactory circuits maintain robust function despite sensory disturbance and how CO2 projection neurons encode activation and sensitization signals. By linking physiology to anatomy through connectome mapping, this work will reveal the functional properties and circuit mechanisms that make host-seeking behavior so remarkably robust in an animal that represents a major global health burden.

PS2.37

Vision and photoreception (Part 1)

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Strict photoreceptor mosaics in the retina of the night-migratory Eurasian blackcap
Night-migratory songbirds navigate over vast distances with remarkable precision using multiple environmental cues, including the Earth's magnetic field and potentially polarized light patterns. It is thought that the light-dependent magnetic compass sense and polarization vision originate in double cone (DC) photoreceptors of the retina. DC comprise an accessory and principal member, which have recently been termed PR5 and PR6, respectively. Theoretical considerations suggest that a highly ordered spatial arrangement of neighbouring DCs could facilitate the extraction of magnetic and/or light polarization information independently of natural variations in light intensity. In this study, we investigated the distribution and spatial organization of cone photoreceptors in the retina of the night-migratory Eurasian blackcap (*Sylvia atricapilla*) using flat-mount preparations. Oil droplets, which serve as light-filtering structures in avian cones, exhibit different spectral properties depending on the cone type. Thus, cone types were distinguished by utilizing the characteristic fluorescence of their oil droplets. In addition, cones were immunolabeled for their respective opsins. In the central retina, DCs exhibited a strikingly regular organization. Individual DCs were oriented at approximately 90∞ relative to their nearest neighbours, forming a strict square mosaic. Notably, a single ultraviolet-sensitive cone (PR4) was consistently located at the centre of each DC square, while red-, green- and blue-sensitive cones (PR1, PR2, PR3, respectively) surround the squares. This strict square mosaic is reminiscent of the highly ordered cone photoreceptors in the fish retina, which allow for polarisation vision. Whether this is indeed the function of the highly ordered spatial arrangement of cone photoreceptors in songbirds remains to be seen. This study was funded by SFB 1372 'Magnetoreception and Navigation in Vertebrates'.

PS2.91

Learning, memory and cognition

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Scanning and active sampling behaviours emerge from conserved insect neural circuits
Navigating insects often pause and rotate to sample their surroundings, behaviours termed scanning. These and other active sampling behaviours embody navigational uncertainty, and are key for spatial learning, yet their neural basis remains unclear and existing models impose scanning behaviours rather than explaining its emergence. Here, we show that desert ants' scanning dynamics can emerge spontaneously from the same conserved neural circuits used for goal-directed navigation, without requiring a specialized scanning module. We built a biologically grounded model combining central complex (CX) steering and lateral accessory lobe (LAL) oscillators, and added a downstream stochastic inhibition of forward speed. This minimal system produced diverse, realistic scan dynamics; saccades, fixations and reversals, whose features were qualitatively compared to high-speed video recordings of Melophorus bagoti scanning. Detailed analysis of these natural scans confirmed model predictions, including how scan structure depends on oscillator

phase, goal-heading deviation, and navigational uncertainty. Furthermore, the model reveals that simple modulation of forward speed unifies a broad range of behaviors across ant species, from dashes to smooth oscillatory trajectories to pirouettes and voltes. Crucially, it establishes a distributed control principle, where forward speed acts as a single adjustable parameter, for both individuals and through evolution, to regulate the balance between goal-driven exploitation and information-seeking exploration.

PS1.7

Motor systems, sensorimotor integration and behavior (Part 1)

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Redesigning locomotion: remodeling of proprioceptors after injury in the medicinal leech

Information from the brain to pattern-generating circuits is often required for the organization of rhythmic motor behaviors. Without such descending input, in the medicinal leech Hirudo verbana, locomotor performance is disrupted. Remarkably, several weeks following a nerve cord transection, leeches recover their full ability to crawl. This recovery is not mediated by regrowth of central connections but appears to rely on the remodeling of peripheral sensory neurons. How crawl circuits become newly reliant on this afferent input for the restoration of oscillator-to-oscillator coupling is not known. Here, we studied the morphology of the eight bilaterally paired stretch receptors (SRs) (i.e., proprioceptors) that monitor the positions of each body segment. We labeled SRs with a 3000 MW tracer (MicroRuby) in control and crawl-recovered animals. We examined SR terminal arbors in the ganglion just posterior to the transection site and those in more distal ganglia. In uninjured animals, the SR's axonal terminals did not project past their segment of origin. In crawl-recovered animals, however, we observed that SRs grew additional processes that projected intersegmentally with novel terminal processes and boutons in adjacent ganglia. Interestingly, one type of SR never exhibited new growth, which was correlated with its developmental pathfinding ability. Each of the SRs also appears unique with some sending projections only anteriorly, and others sending projections both anteriorly and posteriorly. These results suggest that remodeling of crawl circuitry after injury could be directed by a few key proprioceptors. We are currently exploring whether the extent of SR growth, housing environment, and motivation to crawl are associated with the degree of crawl recovery through automated video analysis. Together, our findings establish the leech as a model for examining how a centrally-driven locomotor system becomes newly dependent on peripherally-driven inputs.

PS1.97

Mechano- & thermoreception

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Variations in touch representation in the avian forebrain
Somatosensation is essential for animals to perceive the external world through touch, allowing them to detect physical contact, temperature, pain, and body position. Studies on rodent vibrissae have highlighted the organisation and processing in mammalian somatosensory pathways. Comparative research across vertebrates is vital for understanding evolutionary influences and ecological specialisation on somatosensory systems. Birds, with their diverse morphologies, sensory abilities, and behaviours, serve as ideal models for investigating the evolution of somatosensation. Prior studies have uncovered tactile-responsive areas within the avian telencephalon, particularly in pigeons, parrots, and finches, but variations in somatosensory maps and responses across avian species are not fully understood. Here we explore somatotopic organisation and neural coding in the telencephalon of avian species with different feeding and flight behaviours using in vivo extracellular electrophysiology. We recorded activity in response to controlled tactile stimuli on various body regions, including airflow on the wings. These findings reveal unique representations of body regions across distinct forebrain somatosensory nuclei, indicating significant differences in the extent of areas dedicated to certain body surfaces, which may correlate with their behavioural importance.

PS3.84

Olfaction, taste and chemical sensing

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Egg-laying behaviour and sensory ecology of a coastal rockpool mosquito (*Aedes togoi*)
Mosquitoes typically prefer to lay eggs in and around freshwater because high salinity pools can be lethal to their aquatic offspring. Some species, however, have adapted to laying eggs in saline water. *Aedes togoi*, the coastal rockpool mosquito, readily lays eggs in and around concentrated seawater pools in Southeast Asia and the Pacific Northwest. Their larvae are adapted to frequent fluctuation of salinity, temperature, and volume in these pools. Salt is a potent aversive egg-laying cue in widespread disease vectors, like the yellow fever mosquito *Aedes aegypti*. Using quantitative behavioral assays and transcriptomic approaches, I aim to address the following questions:
1) What components of seawater are attractive and aversive to *A. togoi* when egg-laying?
2) What sensory appendages and molecular receptors do they use to sense these components? 3) What controls diapause at the egg and larval stages?

This will lay the groundwork for the future investigation of molecular mechanisms underlying this resilient mosquito's behaviour and create opportunities for comparison with related disease vectors. If shared mechanisms are identified, this work could shed light on how mosquitoes of greater public health risk lay eggs and why few mosquito species are fit to live in saline environments.

PS1.95

Mechano- & thermoreception

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Tracking the invisible - hydrodynamic trail following in pinnipeds

The vibrissae of pinnipeds constitute a highly sensitive hydrodynamic receptor system enabling them to detect minute water disturbances left behind by prey. These disturbances, known as hydrodynamic trails, can persist for several minutes and extend over considerable distances. Behavioral studies have demonstrated that harbor seals (*Phoca vitulina*, Phocidae) and California sea lions (*Zalophus californianus*, Otariidae) are capable of following hydrodynamic trails left by a miniature submarine. Increasing delays between trail generation and trail following negatively affected tracking performance; harbor seals, which possess undulated vibrissae, were able to track older trails than California sea lions, whose vibrissae are smooth in outline. These differences in tracking performance were attributed to morphological variations in vibrissal structure and the associated detection mechanism. However, since the submarine-generated trails may dissipate more rapidly than the long-lasting trails of fish, the maximum age of detectable natural trails remained undetermined. To address this, in the present study fish-like trails were generated using a soft plastic swimbait (27 cm) attached to a fishing rod, and the maximum delay at which harbor seals and California sea lions can reliably detect and track these trails under seminatural conditions was investigated. The resulting trails were analyzed using particle image velocimetry. The results show that harbor seals can detect and track trails corresponding to distances exceeding 130 m from the swimbait, as estimated from trail age and swimming speed. In contrast, California sea lions tracked such trails over significantly shorter distances. Moreover, following trail loss, only harbor seals were able to relocate the trail after a period of searching. These results highlight the species-specific differences in long-distance prey detection and their potential ecological relevance.

PS3.11

Motor systems, sensorimotor integration and behavior (Part 3)

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Representation of visual motion uncertainty in an insect neck connective

Sensory inputs are typically noisy, sparse, and uncertain: they fluctuate from one moment to the next, often containing a limited sample of available information. One general solution that the brain uses to generate behaviorally relevant response is to integrate information over time and trigger a motor command once accumulated evidence reaches a threshold. The random dot kinematogram (RDK), in which a subset of dots moves coherently while the remainder move randomly, enables precise control over motion uncertainty and behavioral difficulty. This stimulus has been traditionally used in primates and rodents to understand the neural underpinnings of perceptual decision making. Recent work in larval zebrafish have shown that innate optomotor responses become stronger as motion certainty increases. Evidence accumulation underlying this response localizes to the anterior hindbrain, an evolutionarily conserved region distinct from the primate lateral intraparietal

cortex. Flying insects also show strong optomotor responses to wide-field motion. Here we test whether the compact brains of insects are likewise able to represent and respond to uncertainty in wide-field motion cues, potentially revealing conserved principles or novel solutions for perceptual decision making. We adapted the RDK paradigm for the large agile hawkmoth *Manduca sexta*, which requires fast active stabilization of wide-field motion during hovering in the dark. Tethered moths viewed RDKs at five coherence levels (0-100%) using a 2-Hz triangular velocity profile while extracellular recordings were made from the ventral nerve cord, an information bottleneck between brain and periphery. Across 101 recorded units from nine moths, 25 exhibited monotonically increasing, phase-locked responses with stimulus certainty. We hypothesized a step-function-like threshold, but these units instead showed variable tuning with coherence: 68% sigmoidal, 12% quadratic, 12% exponential, and 8% linear. Permutation tests additionally revealed 22 units lacking optomotor directional tuning but with responses scaling with coherence alone. These populations encoding stimulus certainty together with wide-field motion-sensitive units, could underlie control of motor gain and gating of sensory-motor responses according to motion reliability.

PS3.2

Motor systems, sensorimotor integration and behavior (Part 3)

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Neuroendocrine regulation and persistent effects of hypergravity exposure on geotaxis and activity in *Drosophila melanogaster*

Gravity, a constant force throughout evolution, has fundamentally shaped biology, playing a critical role in locomotion, balance, and orientation across species - from unicellular organisms to complex multicellular life. Despite its pivotal role in biomechanics and physiology, how gravity affects different aspects of locomotion and activity remains less understood. Using a hypergravity simulator, we found that acute exposure to 4g impairs spontaneous climbing behavior in *Drosophila melan*ogaster, while startle-induced climbing remains unaffected when tested at 1g. After exposure to higher gravity levels (7g, 10g, and 13g), spontaneous climbing deficits become more pronounced at 1g, yet startle responses remain largely intact. Strikingly, we found that a single 24-hour exposure to moderate hypergravity (4g) induces a robust and long-lasting increase in locomotor activity that persists for more than 40 days, indicating long-lasting effects of hypergravity. In parallel, whole-body triacylglyceride measurements revealed gravity- and time-dependent modulation of energy stores following hypergravity exposure. To explore neuroendocrine regulation of our hyperactivity phenotype, we focused on octopamine, a conserved neuromodulator associated with arousal and locomotor activation in invertebrates and considered a functional analogue of vertebrate noradrenaline. Octopamine mutants showed no hyperactivity following 24 hours of hypergravity exposure compared to genetic controls, suggesting that octopamine, and its associated effects on activity and energy use, are likely key players in modulating activity under altered gravity. Together, our findings demonstrate that altered gravity exposure modulates locomotion, with lasting consequences for activity and energy homeostasis after return to 1g. Furthermore, we are beginning to identify how these effects occur through alterations in neuroendocrine regulation.

PS2.82

Learning, memory and cognition

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A closed-loop virtual reality approach to study context-dependent memory in *Drosophila*

Foraging animals must continuously balance the drive to explore for food with the urgency to avoid danger. A well-studied example is memory-guided local search, in which animals loop around a discovered food source, demonstrating that learned valence can directly shape navigational strategy. In natural environments, however, animals must simultaneously weigh attraction and avoidance, adjusting their behavior based on internal state, experience, and context. How the brain integrates these opposing valence signals into coherent spatial navigation remains a fundamental question in circuit neuroscience. *Drosophila* provides a powerful genetic toolkit for this analysis: targeted genetic access, functional neuronal imaging, and a complete connectome. The Mushroom Body is the brain's primary center for associative learning via dopamine-driven plasticity. The Central Complex computes head direction and generates steering commands. Yet how these regions coordinate to integrate competing memories into flexible navigation remains an open question. Here we present a closed-loop virtual reality approach for tethered flies to characterize context-dependent aversive memory to guide spatial navigation. The system delivers visual scenes and aversive stimuli with ultra-low latency, updating the virtual environment in closed loop based on the animal's heading, speed, and position. A side-view camera tracking leg movements and posture enables precise quantification of behavioral responses during memory-guided tasks. We condition flies to associate visual stimuli (colors, pattern orientations) with laser-induced heat punishment and are characterizing the resulting avoidance trajectories. In future work, we will integrate this behavioral VR paradigm with two-photon calcium imaging to simultaneously measure learned memory traces and behavioral navigational responses. This combination will allow us to dissect the neural circuits that transform context-dependent memories into goal-directed spatial navigation.

PS2.45

Auditory systems and acoustic signaling (Part 2)

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The impact of the GABA receptor on the mosquito *Anopheles coluzzii* hearing

Hearing is an important sense for *Anopheles* mosquitoes, particularly regarding the mating behaviour within a swarm. Male mosquitoes commonly locate females acoustically (phonotaxis) to copulate by tracking sounds emitted during flight. The auditory sensory organ in mosquitoes is the antennae, consisting of two components: the flagellum, which detects sound, and Johnston's organ (JO), which is the auditory organ. The hearing process is controlled by an extensive neuromodulatory network in the antennae which includes the GABA_A receptor known as the Resistance to Dieldrin Locus (RDL), which is the target of many neurotoxic pesticides like Fipronil and some organochlorines (e.g. dieldrin). In this study, we compare the auditory function of two *Anopheles coluzzii* - Tiefora lineages that differ by a mutation in the RDL (wt 296A vs. resistant A296S), in order to investigate the effect of the mutation on the auditory pathways. We also discuss this auditory data in relation to the molecular structure of the RDL by comparing the trans-membrane channel width of the different RDL variants, as well as their binding strengths to dieldrin. Mosquitoes were maintained in the insectary under controlled conditions of 12h:12h light/dark cycle with 1-hour ramped light transition to simulate dawn/dusk, temperature (28∞C) and relative humidity (80%). Laser Doppler Vibrometer (LDV) directed to the tip of the flagellum to infer the flagellum's mechanical state and the auditory tuning to analyse the auditory function in both lineages. Moreover, we are using the Hole2 program to investigate the RDL channel widths on the homology models built using Modeller. The binding strength of dieldrin is obtained using Autodock. We hypothesise that this mutation affects hearing sensitivity and, consequently, mating behaviour.

PS1.15

Motor systems, sensorimotor integration and behavior (Part 1)

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Interplay of subcortical and cortical regions in the control of zebra finch call interactions

Vocal turn-taking is a ubiquitous form of social interaction across many species, including humans. Zebra finches form an excellent model to study the neural bases of this coordinated behavior, as they are highly vocal and engage in nearly continuous call exchanges with conspecifics. Previous studies have implicated the cortical premotor nucleus HVC in the temporal control of call interactions, while the subcortical area dorsomedial nucleus of the intercollicular complex (DM) has been linked to the production of vocalizations. Although both areas form part of a recurrent network critical for calling behavior, it remains unclear how their interactions contribute to the control of turn-based vocal exchanges. We address this question by examining the functional connectivity between subcortical (DM) and cortical (HVC) brain regions. For this, we electrically stimulate DM in anesthetized birds while recording from the premotor nucleus HVC using high density Neuropixels 2 probes. Conversely, we stimulate HVC and record from DM to assess bidirectional interactions. In addition to this, stimulation experiments in DM reveal potential contributions of the region towards shaping the acoustic structure of calls. Preliminary

results using microstimulation indicate that DM activity influences the duration of calls used in vocal exchanges. Taken together, these experiments provide a systems level analysis of how subcortical and cortical circuits influence each other to regulate vocal behavior and its context-dependent use during social communication.

PS2.15

Motor systems, sensorimotor integration and behavior (Part 2)

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Adaptive flight strategies for challenging light environments in a nocturnal hawkmoth
Flying insects use visual information to control and stabilize their flight. In nature, this information is highly dynamic and thus presents a challenge for insect brains to extract reliable and relevant information from it. And yet, many animals master this challenge on a daily-and nightly-basis. Understanding this ability requires studying both sensory processing and behaviour, which act in concert: as senses guide an animal's movements, the movements shape the sensory input. In flying insects, alterations in flight speed and distance to objects shape the spatiotemporal frequency composition of their visual input. Here, using the nocturnal hawkmoth *Deilephila elpenor*, we studied how these flight alterations are adjusted to support visual acquisition in dim light. In dim light, the visual system becomes coarser and slower to increase sensitivity. We therefore expect animals to decrease their flight speed in dim light to bring the spatiotemporal frequency composition of their visual input within the range of their visual system's adaptations. To record the moths' flights, we used a large-scale motion capture system (12x11x6m), where animals could choose their flight strategies flexibly and use their full repertoire of flight manoeuvres. We matched light intensities to three natural conditions within the moth's visual activity range (starlight, moonlight, and twilight) and presented them either as ambient light or as quickly changing sequences (20- or 10-minute intervals). We found that the animals did not adjust their flight speed in the ambient light intensities but instead achieve high flight speeds even in starlight. However, when light intensities changed rapidly, animals initially slowed after decreases in light intensity and sped up after increases but gradually recovered their flight speed. Finally, we radiometrically analysed visual information in the flight arena to propose mechanisms behind the unexpectedly high flight speeds at the lowest light levels.

PS1.48

Social behavior and neuromodulation (Part 1)

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Behavioral evidence and neuronal correlates of the Franssen Effect in gray treefrogs
The ability to perceive and localize sounds is fundamental to many organisms and plays a critical role in survival and communication. Auditory illusions, such as the precedence effect (PE) and Franssen effect (FE), reveal how the brain resolves spatial and temporal discrepancies in acoustic input. In the PE, subjects perceive 2 identical sounds, differing by a few milliseconds in onset time and broadcast from 2 spatially separated loudspeakers as both coming from the source of the leading sound. The FE involves a transient fast-rise (TFR) tone followed by a slow-rise, long-duration (SR-LD) tone from another speaker; the SR-LD tone is mislocalized to the source of the leading TFR tone. Unlike the PE, studying the FE in animals poses the challenge of ascertaining that the subject actually perceives the SR-LD tone as originating from the transducer broadcasting the leading TFR stimulus. Thus far, suitable behavioral assays have been lacking. To address this gap, we studied the North American gray treefrog, *Hyla versicolor* (Hv), which produces advertisement calls that have SR-LD pulses; females strongly prefer calls with slow-rise pulses over fast-rise alternatives. We leveraged this natural preference to create a behavioral assay for the FE. In phonotaxis experiments females approached the speaker broadcasting TFR pulses when SR-LD pulses from another speaker were 0-10 ms delayed, but not when advanced. Hv females, therefore, mislocalized the SR pulses to the source of the FR pulses. confirming that they experienced the FE. To identify neural correlates of the FE, we recorded from auditory midbrain neuronsin vivo. In a subset of neurons, responses to lagging contralateral SR-LD pulses were strongly suppressed when ipsilateral TFR pulses were temporally advanced, closely mirroring the behavioral illusion. Together, these findings establish gray treefrogs as a powerful model for investigating the neural basis of the FE and related auditory illusions.

PS2.98

Metabolism, biological rhythms and homeostasis

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Neurophysiological mechanisms of thirst suppression during hibernation
Thirteen-lined ground squirrels hibernate for 6-8 months of the year without access to water. During this time, they remain within their burrows and cycle between two physiological states: torpor and interbout arousal (IBA). Torpor is a period of inactivity and low body temperature that approaches ambient levels (2-4∞C). Bouts of torpor are punctuated by spontaneous ~24-48-hour IBAs when squirrels enter an active-like state with a body temperature of 37∞C and move around in their burrows, but do not leave in search of water. Even when water is provided, squirrels in IBA refuse to drink, indicating that drinking behavior is inhibited during hibernation. That these squirrels can endure such a long period of time without drinking represents a remarkable physiological phenomenon, but the molecular mechanisms are unknown despite more than one century's worth of hibernation research. Here, we show that even though squirrels show peripheral and behavioral signs of fluid deficiency during hibernation, they have blunted sensitivity to angiotensin II and blood osmolality - two potent thirst cues. Furthermore, we show that thirst neurons located in SFO and OVLT can bind angiotensin II and respond by increasing intracellular calcium. Nevertheless, they fail to initiate sustained activity when challenged with acute dehydration, suggesting reduced excitability. Indeed, we show, using patch-

clamp electrophysiology, that thirst neurons are hyperpolarized and exhibit increased sensitivity to inhibition by GABA, supporting the notion that the subcortical nuclei responsible for initiating drinking behavior are tonically inhibited during hibernation. Our work thus explains how mammalian hibernators can tune their sensitivity to interoceptive cues to survive without water for several months and reveals a remarkable plasticity of the neurophysiological pathway that controls thirst in mammals.

PS2.18

Motor systems, sensorimotor integration and behavior (Part 2)

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Goal-directed behavior: nest building in a shell-dwelling cichlid *Lamprologus ocellatus*

Goal-directed behaviors involve the flexible organization of action sequences to achieve a specific outcome. This requires knowledge of the causal structure of the environment (a world model), allowing organisms to evaluate outcome value and generate appropriate action sequences (Pezzulo et al., Trends Cogn. Sci., 2014). *Lamprologus ocellatus* (LO) is a cichlid, originally from Lake Tanganyika, that breeds in nests constructed from abandoned snail shells. Nest building involves a sequence of stereotyped behavioral motifs, including placing the shell vertically into the substrate and covering it with sand (Grätsch et al., Curr. Biol., 2026). Because reproduction depends on access to such species-typical nests, nest building is a robust and reproducible behavior, making it powerful model system to investigate the neuroethological underpinnings of goal-directed behavior in a lab setting. In this study, we challenge this behavioral program by testing its flexibility in response to environmental manipulations, including changes in shell geometry, substrate properties, and nest state. To examine LO's knowledge of the causal structure of the environment, we present the fish with challenges that prevent immediate nest construction: shells may be obstructed, placed on solid substrate, or positioned on a slope, requiring the fish to remove the obstacle or maneuver the shell to sandy substrate before nest building can proceed. In our previous studies, we identified excitatory and inhibitory neurons in the dorsolateral telencephalon, a hippocampus homologue, that are active during nest building. We now build on these findings by using third-generation in situ hybridization chain reaction and DiI-based neuronal tracing to investigate which brain regions contribute when fish solve experimentally imposed challenges. Together, this work uses the robust nest-building behavior of LO to investigate the neural and cognitive mechanisms underlying goal-directed behavior.

PS1.36

Social behavior and neuromodulation (Part 1)

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Measurement of brain and plasma steroids in a paternal fish via LC-MS/MS

Parental care is a fundamental social behavior that enhances offspring survival and reproductive success. Although paternal care is rare among terrestrial vertebrates, it is more common in fish. Male plainfin midshipman (*Porichthys notatus*), a seasonally breeding teleost fish, provide exclusive parental care, engaging in nest guarding and egg oxygenation. Because males simultaneously invest in courtship and parental care, they face trade-offs in resource allocation during breeding. In mothers, sex steroids are key neuromodulators of parental care. While the maternal brain has been extensively studied, the neuroendocrine mechanisms underlying courtship and care in paternal systems remain poorly understood. Here, we developed and validated a novel extraction and quantification method to measure steroids (e.g., testosterone, 11-ketotestosterone, progesterone, estradiol, and cortisol) in teleost brain and plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We applied this method to quantify steroids in plasma, preoptic area, and telencephalon of males collected from nesting sites in the field across three reproductive states: courting, early paternal care, and late paternal care. In plasma, major active androgens and estrogens, such as 11-ketotestosterone and estradiol, were highest in courting males. In brain, androgens and estrogens were elevated in courting and late paternal males relative to early paternal males. Moreover, progesterone was detected exclusively in courting brain, while 20--hydroxyprogesterone, a progesterone metabolite that supports paternal care in rodents, was exclusively detected in paternal brain. Plasma steroid levels thus do not necessarily reflect brain steroid levels, emphasizing the importance of measuring steroids in specific brain regions. Broadly, this work provides novel insights into the conserved neuroendocrine mechanisms underlying reproductive social behaviors, with comparative relevance to mammals and birds.

PS2.25

Vision and photoreception (Part 1)

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Responses of a locust motion-sensitive neuron to object motion in the rear visual field

Escape behaviours in locusts are evoked by visual neurons that encode images of an approaching object. The lateral giant movement detector (LGMD) and the post-synaptic descending contralateral movement detector (DCMD) together identify the expanding edges of approaching objects across the locust's eye and synapse onto appropriate motor elements for flight steering. The locust eye samples a 360∞ field of view around the animal's body, allowing it to see objects approach from nearly any direction. While many studies investigated encoding of objects in the frontal visual field, there is less information about objects approaching from the rear, which is important to understand how locusts respond to threats from behind. We presented 19 stimuli consisting of projected spheres on different translational, looming, or translation to looming courses to 19 locusts. Initial observation of the DCMD response suggests that the peak firing rate occurs later relative to time of collision compared to objects approaching in the frontal visual field. We also observed a larger delay between the transition of the object from translating to looming when the stimulus originates from the rear visual field. These findings suggest a feature of the neural circuit may result in delays of approaching objects approaching from behind.

PS1.64

Spatial orientation and navigation (Part 1)

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Tracking individual monarch butterfly movements and behaviors in the wild: advances with mSAIL

Monarch butterflies are renowned for their annual trans-continental migration where they congregate in the millions in the oyamel fir forests of central Mexico. Individual butterflies make a single trip to these overwintering sites, and thus monarchs must possess a genetically encoded spatiotemporal program to locate these sites with such regular precision. Decades of tracking work (sticker tagging, stable isotope studies, etc.) have revealed important information about monarchs' migratory paths such that broad pathways are known. More specific information about their daily behaviors and paths during migration, paired with information of the specific environment that they experience, are needed to answer important questions about their navigation strategy and habitat use. We are developing mSAIL, a milligram-scale environmental geolocator for tracking monarchs during their migration. The system is designed to record and compress light and temperature data during the migration while harvesting solar energy for energy autonomy and to wirelessly transmit the data at the overwintering site in Mexico. Daily location of the butterfly is estimated from these data using a deep learning-based localization algorithm. Initial proof-of-principle tests demonstrate the feasibility of mSAIL to record monarch movements over long distances (~800 km) while inferring behavioral modes along the migratory path. Altogether, we have made important progress toward not only measuring continental-scale monarch migration movements, but also understanding environmental influence of migratory behavior.

PS1.49

Social behavior and neuromodulation (Part 1)

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Multimodal communication during snake mobbing in the Arabian babbler: integrating visual displays and vocal signals

Predator mobbing is a collective antipredator behavior with immediate consequences for survival, in which animals approach a threat while simultaneously coordinating calls, movements, and body postures. These signals can serve multiple functions, such as alerting conspecifics, deterring the predator, and recruiting allies, and are directed at

multiple receivers, including the predator itself and group members. In Arabian babblers (*Argya squamiceps*), snake mobbing involves stereotyped "zwick" calls and conspicuous wing-lifting displays, making it a tractable system to examine how vocal and visual signals are structured and integrated across social contexts. We elicited snake-mobbing events using dummy vipers and recorded babblers with a synchronized acoustic camera and high-resolution video camera, enabling individual caller identification, postural analysis, and call localization in the wild. This yielded 3,199 individually identified calls from 52 individuals across 10 groups. Wing-lift displays increased significantly during circling compared to approach, while call rate changes were sex-specific: females increased calling during circling, whereas males and juveniles showed no change. Group size negatively predicted call rate, juveniles performed more wing-lifts than adults, and dominant individuals were more likely to perform silent wing-lifts than subordinates. Calls during wing-lifts were characterized by shorter inter-call pauses and a subtle shift toward lower frequencies and longer durations. Vocalizations and wing-lifts appear to serve partially distinct functions, with calls coordinating conspecific responses and wing-lifts likely directed at the predator. Dominant individuals may focus their display on the predator while subordinates simultaneously call and display more, potentially advertising participation. The acoustic shift during wing-lifts may reflect changes in body cavity resonance during display, and increased call rate likely signalling urgency.

PS3.42

Social behavior and neuromodulation (Part 2)

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A kaleidoscopic view of alloparental behavior in the clonal raider ant

An approaching predator generates a distinct temporal sensory signature, a looming stimulus, alerting a prey. Although the mechanisms for visual looming detection have been thoroughly investigated, those associated with aerodynamic loomingóair movements induced by an approaching predatoróremain largely unexamined. Given that both types of looming involve a non-linear increase in certain signal characteristics with decreasing distance, we tested whether the mechanisms of perception of aerodynamic looming are analogous with those of visual perception. We stimulated the cercal system of the cricket, a well-established model for airflow sensing, with spider-mimicking translating spheres. The size and velocity of the looming object, the two critical parameters in visual looming perception, significantly enhanced the activity of wind-sensitive interneurons and the escape reaction. Moreover, the timing of peak firing occurred after a small delay after crossing a flow threshold, a physiological property typical of visual looming-sensitive neurons. These findings suggest the existence of convergent computational principles of looming detection across different sensory modalities.

PS1.84

Evolution and development

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Parent–offspring conflict and sibling competition in the big brown bat
Parent-offspring conflict occurs when there is a disagreement over parental resource allocation. Sibling competition occurs over allocated parental resources, such as food. Big brown bats (*Eptesicus fuscus*) are an interesting model to study both conflicts because in western North America they give birth to a single pup whereas in the east they give birth to twin pups. We assessed parent-offspring conflict and sibling competition in a captive breeding colony of *E. fuscus* by recording maternal care behaviours in mothers (n = 16) and solicitation behaviours in pups (n = 32)-first with one pup and then with a sibling-over the first 30 days of life. Mothers initiated fewer maternal interactions as pups aged. Moreover, pups required more attempts to attach to their mother's teat as they aged. Interestingly, moms initiated care more frequently when they were presented with both of their pups compared to a single pup. The presence of a sibling also made it more difficult for a pup to attach to its mother's teat, but only early in development. We then experimentally manipulated pup health status (n = 23) to determine how changes in body temperature or feeding status affected the temporal and spectral qualities of the isolation calls that pups emit to solicit maternal care. We manipulated body temperature by placing detached pups in a warm and cold arena (order randomized). We manipulated hunger status by isolating pups for 3-hrs and re-recording their calls in the warm arena. Pups in the cold arena emitted longer duration isolation calls compared to the warm arena. The effect of hunger status is being analyzed. Consistent with parent-offspring conflict theory, our preliminary data reveal that parental investment declines over development, and that moms differentially allocate maternal care in the presence of one versus two pups. The body temperature and hunger status experiment aims to determine if the information contained in isolation calls is an honest signal of pup need.

PS3.49

Vision and photoreception (Part 2)

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Brains, eyes, and bioluminescence: comparative neuroanatomy in Ostracods
Ostracods are a diverse class of minute crustaceans found in nearly every aquatic habitat, from the deep sea to vernal pools, with a wide diversity of sensory ecologies. Within the family Cypridinidae, ostracods have evolved bioluminescent behaviors including defensive anti-predator displays and in the sub-tribe Luxorina, luminescent courtship displays, earning them the nickname 'sea fireflies' (Morin, 1986; Ellis et al., 2023). These displays present behavioral challenges that may be reflected in brain and visual system complexity. Many species live in sympatry with overlapping displays, requiring females to distinguish species-specific patterns and intercept desired conspecifics in a noisy visual environment (Gerrish and Morin, 2016). Males of some species are also known to flexibly adapt their signaling strategy, choosing to initiate, entrain on, or intercept displays (Rivers and Morin, 2009), demonstrating remarkable behavioral plasticity, the neural basis of which is unknown. This study provides the first neuroanatomical descriptions of multiple ostracod species, including non-luminous, luminous, and luminous-signaling cypridinids. We aim to determine how the eyes and brains of ostracods vary with sensory ecology, and whether species with luminescent courtship show morphological specialization for these behaviors. Using high-resolution micro-CT scans, we generated 3D reconstructions of the compound eyes and brains of these species. We also segmented two sensory integration structures,

the mushroom bodies and central complex. Quantitative measurements of eye traits, including size and organization of ommatidia, and brain substructures, along with phylogenetically informed statistics allow comparisons of visual and neural investment across species. This work advances our sparse knowledge of ostracod neuroanatomy and deepens understanding of how sensory ecology and species-specific behaviors influence the evolution of sensory systems and brains.

PS2.41

Vision and photoreception (Part 1)

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Allometric scaling of visual resolution in compound eyes of bumblebees, *Bombus terrestris*

Body size variation within species can impact sensory system performance. However, size-dependent differences in vision remain poorly understood. *B. terrestris* workers exhibit remarkable intracolonial size variation while performing similar visually guided foraging tasks. Previous studies suggest larger workers have enhanced visual capabilities, but direct physiological evidence has been lacking. We investigated how body size shapes visual resolution and contrast sensitivity across the complete size spectrum of *B. terrestris*, from small in-hive workers (ITD < 4 mm) through medium foragers (ITD 4-6 mm) to large foraging queens (ITD > 6 mm). Using intracellular electrophysiological recordings from 49 photoreceptors, we measured receptive field properties and responses to targets ranging from 0.3∞ to 15∞ to determine the minimum detectable target size. We combined these physiological measurements with optical measurements and morphometric analysis. Eye area and facet diameter showed negative allometry with body size (exponents 0.67 and 0.56, respectively). Photoreceptor acceptance angles (??) decreased dramatically with body size, from 3.68∞ in small workers to 1.49∞ in queens (m=-0.33, R≤ = 0.50), allowing queens to resolve smaller objects (0.7 versus 1.5∞). Contrast thresholds decreased only modestly with body size (m=-0.06, R≤ = 0.26), suggesting that the main improvement in visual performance results from larger facets rather than improved contrast sensitivity per se. Pseudopupil based optical mapping across the visual field revealed a strongly anisotropic frontal acute zone, consistent with the eye's elongated morphology. Minimum vertical interommatidial angles (??V) reach 0.7∞ between -10 and +10∞ elevations. In contrast, horizontal interommatidial angles (??H) were similar across most of the frontal visual field, at around 2.5∞. Together, these parameters are consistent with theoretical for predictions of diffraction limited optical sampling for vertical, but not horizontal resolution. These findings provide the first direct physiological evidence linking eye morphology to visual performance across body sizes, demonstrating that larger *B. terrestris* have superior spatial resolution.

PS3.14

Motor systems, sensorimotor integration and behavior (Part 3)

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MusBioMaus 2.0 - a full body biomechanical model of the mouse
Animals perform a rich behavioral repertoire that requires sophisticated orchestration of numerous muscles over time. Acquiring this coordinated activity from multiple muscles simultaneously presents a major challenge. To this end, musculoskeletal modeling has provided a valuable framework for studying motor output. However, current musculoskeletal animal models are often partial or lack anatomical fidelity. Here we present MusBioMaus 2.0, an anatomically accurate full-body biomechanical model of the mouse for replicating motor behavior from behavioral recordings and generating in silico muscle activity. Using µCT and MRI scans of mice, we segmented an entire mouse skeleton and identified muscle attachment points. Next, we set bone rotational centers and skeletal hierarchy and ported the model into the physics engine MuJoCo. The resulting musculoskeletal model consists of 234 bones distributed across 163 joints, collectively providing 344 degrees of freedom. We used behavioral recordings from freely moving mice to obtain 3D keypoint estimates, which were then constrained to our biomechanical model. Using inverse kinematics, we minimized the distance between video frame keypoints and those constrained to our model, replicating recorded behavior with our model and calculated required muscle activations for the kinematic transformations, allowing us to generate in silico electromyography (EMG) signals. To validate our model, we recorded in vivo EMG-activity of selected muscles using Myomatrix arrays and acquired synchronized video. Extracted 3D keypoints enabled our model to simulate behavior and generate in silico EMG, which we then compared to our experimental data. In brief, our anatomically accurate full body musculoskeletal mouse model can imitate behavior from video recordings and is able to generate in silico muscle activity. We intend to release this model as an open-source tool for the community and expect our methodology to transfer smoothly across species.

PS2.12

Motor systems, sensorimotor integration and behavior (Part 2)

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Sensorimotor circuits for performance and refinement of stereotyped zebra finch flight
Bird flight is an extraordinary example of complex motor control and flexibility. While extensive work has characterized flight kinematics and recent studies have demonstrated that flight behaviors show the hallmarks of skilled motor learning, little is known about the neural circuitry and activity that underlie learning, flexibility, and performance of flight. Here, we used activity dependent protein phosphorylation (pS6) associated with flight training in zebra finches. We imaged pS6 expression immediately after flight in two groups of birds that either received one or five days of flight training, and in a control group that did not fly prior to imaging. We identified regions that were differentially active across these three groups including somatosensory regions in the basorostral forebrain as well as putative sensorimotor regions in the nidopallium, arcopallium, globus pallidus, and striatum. To gain further insight into neural activity patterns during flight, we targeted a region in the dorsorostral caudolateral nidopallium (NCLdr) that showed greater increases in pS6 expression after flight in trained birds. Using fiber photometry with the calcium indicator

GCaMP8s, we characterized population dynamics during flight. Together, these findings begin to elucidate the circuitry supporting flight flexibility and suggest that population activity in regions of the pallium and subpallium, including the NCLdr, may contribute to the initiation and refinement of learned flight behavior.

PS1.60

Social behavior and neuromodulation (Part 1)

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Frontal cortex modulates jamming avoidance in echolocating bats

In acoustically cluttered environments, animals need to adapt their sensory and motor strategies flexibly to navigate and communicate effectively. Echolocating bats are an excellent model to study such adaptive behaviours, as they actively modify vocal output based on incoming auditory information. Here, we examined echolocation behaviour in fruit-bats (*Carollia perspicillata*) swung on a pendulum in silent and noisy conditions. The latter included playback of either of continuous broadband noise, or predictable 4Hz Amplitude-modulated (AM) noise. When exposed to both constant and AM noise echolocating bats used multiple adaptive vocal strategies to overcome jamming. These strategies included increased call sound pressure level, consistent with a Lombard-like response, as well as prolonged call duration. Bats also shifted the spectral structure of echolocation pulses by lowering peak frequency. In AM noise, echolocating bats also aligned call onsets to the quiet phase of the amplitude modulation cycles, demonstrating temporal coordination consistent with adaptive jamming avoidance. To investigate the neural substrates underlying this adaptive vocal behaviour, we performed pharmacological inactivation in the Frontal Auditory Field (FAF) using the GABA agonist muscimol. Inactivation of the FAF did not prevent bats from producing echolocation pulses in the pendulum task but it did disrupt their vocal adjustments to noise. FAF deactivation also weakened the noise-dependent modulation of call timing. Taken together, our findings show that bats use multiple strategies to reduce acoustic interference and highlight frontal cortex as a key component supporting the flexible coordination of adaptive vocal behaviour and active sensing in acoustic environments.

PS1.37

Social behavior and neuromodulation (Part 1)

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Neural mechanisms of efficient collective behavior in zebrafish

A striking example of collective behavior is fish schooling, where animals integrate cues from numerous neighbors to coordinate movement. How the brain manages such computations, often in highly dynamic and cluttered environments, remains largely unknown. We investigated these processes in larval and juvenile zebrafish using behavioral experiments in both naturalistic and virtual reality environments. We find that zebrafish can parse complex social information from simplified visual statistics, namely the retinal occupancy patterns of neighbors. They integrate and average these patterns independently within each eye, then use a ‘winner-take-all’ strategy to combine information across the eyes and guide movement responses. Although genetically encoded, these computations display remarkable flexibility. Social responses occur within milliseconds, yet their gain dynamically adjusts over longer timescales, from minutes to hours, based on recent experience of social density. Using similarly simplified visual cues, zebrafish estimate social density through temporal integration of neighbor-induced looming signals. This mechanism suppresses responses in crowded conditions and enhances them in lower-density environments, yielding a scalable strategy across diverse social contexts. Mathematical modeling confirms that these efficient computations account for collective behaviors across zebrafish development. Using brain-wide imaging at cellular resolution, we identified distinct neural clusters in early visual areas for monocular visual integration, and specialized clusters in the left habenula and hindbrain that implement binocular processing. Together, these results link a compact computational algorithm to its distributed neural implementation and demonstrate an efficient neural mechanism for complex social interactions.

PS1.96

Mechano- & thermoreception

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Cold-induced spreading depolarization as a developmentally regulated neural protective mechanism

Chill coma is a reversible state of paralysis that occurs in insects when temperatures fall below a critical thermal minimum. Neurophysiologically, chill coma is associated with failure of ion homeostasis across neuronal membranes, often involving spreading depolarization (SD) in the central nervous system that silences neuronal activity and disrupts motor functions. This neuronal silence is presumed to be beneficial for surviving the cold stress by limiting ATP consumption, reducing oxidative stress, and preventing membrane damage, making neural damage and subsequent death less likely. Behavioral studies using *Drosophila melanogaster* have demonstrated that chill coma survival is dependent on animal age and developmental stage. For example, larvae of different stages varied in survival rates after cold stress, with third instar larvae being more susceptible to death than first instar larvae. We hypothesize that the reduced survival rate of third instar larvae is caused by a lower susceptibility to SD. We have developed a high-throughput, whole-brain fluorescence imaging assay for *Drosophila* larvae and provide the first evidence of the existence of SD in larvae across all developmental stages due to rapid cooling. Our techniques allow for the observation of SD initiation sites, spread dynamics, and initiation and recovery temperatures. Our data indicate that third instar larvae require exposure to colder temperatures (5.0 ± 0.6∞C; N=10) to elicit SD than first (6.4 ± 0.6∞C; N=10) or second (6.6 ± 0.4∞C; N=10) instar larvae. This is consistent with our hypothesis and suggests that susceptibility to SD may alter survival rates following cold stress. We are currently analyzing spread dynamics to determine whether developmental differences in brain structures influence SD initiation location or velocity. Additionally, we are examining recovery temperatures to determine whether susceptibility to SD alters the animal's ability to regain motor function.

PS1.87

Evolution and development

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Save the nurse, save the hive: Insights in the role of mrjp1 as protective genes in the honey bee brain

The major royal jelly proteins (mrjps) are some of the most highly expressed genes in Western honey bee (*Apis mellifera*) brain and are tightly correlated with the transition between nursing and foraging. These nine novel genes are well known for their nutritive properties in royal jelly; however, the role of they play in both the brain, and the nurse-forager transition, is understudied. We developed a line of mrjp-expressing *Drosophila melanogaster* to study the effects of these genes, and also allowed us to compare results to experiments in honey bees. Expressing mrjp1 in the mushroom bodies created a nurse-like brain genetic state in our flies. Further metabolomic comparisons revealed that these genetic changes create a metabolome that also resembles that of nurse honey bees. Several genes and metabolites present in these analyses have been associated with oxidative stress protection which us to perform follow up assays. Both *Drosophila* and honey bees were exposed to the neurodegenerative herbicide Paraquat (PQ) and were put through a starvation stress assay and a geotaxis behavioral assay. In the starvation assay, mrjp1-expressing groups survived the combination of PQ stress and starvation more than non-expressing groups. Similarly, in the geotaxis assay, mrjp1-expressing groups maintained their ability to climb post PQ exposure compared to their non-expressing counterparts. Taken together, the results of our assays suggest that mrjp1 is creating a protective phenotype in nurse honey bees, allowing them to weather stressors that impact the colony. This insight could shed further light on what niche the mrjps have evolved to fill, while also providing a novel area for translational research.

PS2.77

Spatial orientation and navigation (Part 2)

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Wayfinding as de-differencing: nondecompositional magnetic navigation

There is compelling behavioral evidence that phylogenetically diverse animals use information derived from Earth's magnetic field as a navigational cue. Despite this, characterization of both the sensory structures and processing mechanisms underlying this sense remain largely elusive. Without an understanding of internal representation of magnetic cues, researchers studying this sense must develop an a priori framework for describing and characterizing sensory cues and, importantly, their parameterization. Generally, magnetic cues are orthogonally decomposed into intensity (overall strength of the field), inclination angle (angle between the field and Earth's surface), and azimuth

(horizontal angle of the field). The declination of the field, or its horizontal angle relative to geographic north, is also often used as an additional, relational descriptive parameter. These parameters together capture a complete description of the magnetic field at any point on Earth, but they are each nonetheless one component of an orthogonal decomposition of a fundamentally contiguous, three-dimensional signal. That is, our approach to describing the magnetic field is accurate for generating and describing sensory cues to which animals respond, but may not bear resemblance to how these same cues are represented within animal nervous systems. Indeed, while this kind of signal decomposition is an effective and common strategy in human-engineered systems, animals do not always deploy a similar approach to sensory encoding. Here we explore the possibility that animals might encode the magnetic field as a single contiguous signal and what benefits this approach could confer. In particular, we present the possibility of magnetic navigation as a signal de-differencing problem rather than a problem of complex relational mapping between disparate orthogonal signals, potentially lowering the complexity of processing required for magnetic navigation.

PS3.82

Olfaction, taste and chemical sensing

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Loss of olfaction reduces caterpillar performance and increases susceptibility to a natural enemy

Insect herbivores such as caterpillars are under strong selection pressure from natural enemies, especially parasitoid wasps. Although the role of olfaction in host-≠plant seeking has been investigated in great detail in parasitoids and adult lepidopterans, the caterpillar olfactory system and its significance in tri-≠trophic interactions remain poorly understood. In this study, we investigated the olfactory system of *Pieris brassicae* caterpillars and the importance of olfactory information in the interactions among this herbivore, its host-≠plant *Brassica oleracea*, and its primary natural enemy *Cotesia glomerata*. To examine the role of olfaction, we utilized CRISPR/Cas9 to knockout (KO) the odorant receptor co-≠receptor (Orco). This KO impaired olfactory detection and primary processing in the brain. Orco KO caterpillars exhibited reduced weight and lost preference for their optimal food plants. Interestingly, the KO caterpillars also experienced reduced weight when challenged by the parasitoid *C. glomerata* whose ovipositor had been removed, and the mortality of the KO caterpillars under the attack of unmanipulated parasitoids increased. We then investigated the behavior of *P. brassicae* caterpillars in response to volatiles from plants attacked by conspecific caterpillars and volatiles from plants on which the caterpillars were themselves attacked by *C. glomerata*. After analyzing the volatile compounds involved in these interactions, we concluded that olfactory information enables caterpillars to locate suitable food sources more efficiently as well as to select enemy-≠free spaces. Our results reveal the crucial role of olfaction in caterpillar feeding and natural-≠enemy avoidance, highlighting the significance of chemoreceptor genes in shaping ecological interactions.

PS1.3

Motor systems, sensorimotor integration and behavior (Part 1)

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Neural mechanisms of visually driven action selection in larval zebrafish

To navigate natural visual environments, the vertebrate brain must selectively process the most relevant sensory cue. Visual processing and action selection in mammals involve cortical and subcortical networks. However, the simultaneous recording of multi-region, single-cell level neural activity poses a significant challenge to our understanding of these processes. Here, we investigated the brain-scale neural circuits underlying visually driven action selection in the optically and genetically accessible larval zebrafish. Our behavior model was inspired by the wild teleost from Eno River, Durham, U.S. Here, we tracked fish rapidly switching between hunting and stabilizing themselves against water currents, also known as optomotor response (OMR). In the lab, to investigate how visual stimuli drive action selection, we simultaneously presented larval zebrafish with closed-loop moving gratings and dot stimuli, simulating global motion cues induced by water flow and moving prey. We demonstrated that hunting dominates ongoing OMR-associated behaviors, significantly reducing the animal's response to global visual motion cues. This action selection only biases towards hunting when the dot moves across the binocular visual field. This strong suppression of OMR by the prey-like stimuli remains robust regardless of the stimuli relative directions or novelty, and nevertheless modulated by competing stimulus salience. With simultaneous 2-photon calcium imaging and behavioral tracking in head-embedded zebrafish, we showed that the whole-field motion and dot stimuli are simultaneously represented in parallel in the pretectum and optic tectum. Dot stimulus modulated the response of known OMR circuits in the hindbrain. Overall, we demonstrated a multiregional neural architecture where information from the whole field environment and local objects are parallelly represented and selectively processed for action selection in vertebrates navigating complex sensory environments.

PS3.15

Motor systems, sensorimotor integration and behavior (Part 3)

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The role of an RNA-binding protein in the *Caenorhabditis elegans* motor circuit for backward locomotion

RNA-binding proteins play critical roles in RNA metabolism as cell-type- and developmental-stage-specific regulators. In the nervous system, the architecture and functional specialization of neural networks are strongly influenced by co- and post-

transcriptional regulation of mRNA. While many RNA-binding proteins have been implicated in neuronal development, whether and how their functions are specialized across distinct neural circuits remain unclear. Using the locomotory circuit of the nematode *Caenorhabditis elegans* as a model, I investigate the functions of the RNA-binding protein MBL-1 and its potential specialization across distinct neural circuits. MBL-1 is a homolog of the human Muscleblind-like protein family, which are developmentally programmed regulators of RNA processing, localization, and stability. My current work has uncovered a previously unrecognized locomotory phenotype in mbl-1 loss-of-function mutants. MBL-1 knockout animals fail to initiate body waves from tail to head, which indicates a defect in the neural circuits involved in backward locomotion. I have generated a transgenic strain expressing MBL-1 fused to a green fluorescent protein (GFP) and a chemically inducible degron tag. This strain enables in vivo visualization of MBL-1 protein localization and spatiotemporally controlled protein depletion through a ubiquitin-mediated degradation system, thereby determining the critical neuronal subtypes and developmental stages for MBL-1-dependent locomotory behaviour. GFP-tagged MBL-1 shows high expression in motor neurons and interneurons that mediate and modulate backward locomotion. Based on this expression pattern, I am depleting MBL-1 in these neuronal subtypes to determine where it is required to maintain proper circuit function. Together, my research characterizes the functional specificity of an RNA-binding protein in neural circuits that are essential for navigation and escape behaviours. By identifying the behavioural and circuit-level phenotypes of mbl-1 mutants, my work is building a foundation for circuit-specific transcriptomic profiling to define the RNA targets of MBL-1, which advances our understanding of the molecular logic by which RNA metabolism governs nervous system development and function.

PS3.78

Olfaction, taste and chemical sensing

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Bed bug neuroanatomy: thinking outside the box-spring

Bed bugs are a growing global concern, spreading due to increased human travel and acquired insecticide resistance. Although not known to transmit diseases, these hematophagous insects harmfully affect the tourism industry and psychological health of those who find an infestation in their home (Akhoundi et al., 2023). Even though the presence of the common bed bug, *Cimex lectularius*, has been increasing in society, little is known about their nervous system. Many studies on bed bugs have focused on host-seeking behaviors and their sensory periphery, but brain structures that process host cues remain understudied. Neuroanatomical studies of another species, *Cimex hemipterus*, described the number of axons found within the antennal nerve, their projections to olfactory glomeruli, and the organization of the rhabdomeres of the eyes (Singh et al. 1996). However, other critical structures, such as the central complex or mushroom bodies, as well as identifying their immunoreactivity have yet to be described. To add resolution here, we used immunohistochemistry with antibodies against common neurotransmitters, such as serotonin, dopamine, and GABA, as well as neuropeptides, like allatostatin and FMRFamide, to stain brains of adult *C. lectularius*. We describe their brain anatomy and immunoreactivity with special focus on structures involved in host-seeking including antennal lobes, mushroom bodies and central complex. We compare these to structures in other blood-feeding insects like kissing bugs and mosquitoes.

PS3.24

Social behavior and neuromodulation (Part 2)

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Circulating and central sex steroid variations throughout nest building in a male songbird

Introduction/Aim: Circulating androgens, including testosterone (T) and its precursor androstenedione (A4), play a crucial role in supporting nest building in avian males (e.g., ring doves *Streptopelia risoria*: Martinez-Vargas, 1974; zebra finches *Taeniopygia guttata*: Walters & Harding, 1988; Hébert et al., 2025). Conversion of these androgens into oestrogenic metabolites, such as 17?-oestradiol (E2) is also important (Walters & Harding, 1988), and E2-production ability increases after courtship in the E2-producing preoptic area (POA) of the brain (Dudley et al., 1984). To test whether the steroidogenic pathways through which circulating androgens are produced, as well as preoptic E2 concentrations, change over the course of nest building, we took advantage of the high sensitivity, specificity and multiplicity of Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) to profile circulating and central sex steroid dynamics across the whole nest building period in male zebra finches. Method/Results: Plasma and brains of male zebra finches were collected during early (n = 6) or late (n = 7) nest building. We micro-punched six brain areas, including the POA, and measured sex steroid concentrations using LC-MS/MS (QTRAPÆ 7500). Circulating T remained high throughout nest building despite a drop in its precursor A4. In the POA, E2 concentrations were higher in late compared to early nest builders. No differences were found in other brain regions investigated (i.e., ventromedial hypothalamus, septum, optic tectum, caudomedial nidopallium and cerebellum). Conclusion: Preoptic E2 production increases over the course of nest building and we suggest that male nest building might act as a courtship display in zebra finches. Work funded by the BBSRC (BB/Y002121/1; to SDH, NZH & SLM), Marie Skodowska-Curie Actions (101024039; to MH) and a BSN project grant (to MH). Conducted with local ethical permission (Approval number:BL16544).

PS2.47

Auditory systems and acoustic signaling (Part 2)

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Nightingales categorize acoustic signals to tailor their vocal responses

Rapid and accurate interpretation of acoustic signals in different behavioral contexts is essential to producing an appropriate behavioral response. In songbirds, different vocal categories convey distinct social information, such as territorial defense or mate attraction. The common nightingale possesses a large vocal repertoire that is flexibly deployed during the breeding season in spring, when males need to simultaneously defend territories in nocturnal counter-singing duels and attempt to attract visiting females. While unable to sing, females use vocal signals, such as so called ‘huit’ calls, to indicate their presence to singing males. These calls may function as contact or luring signals. For males, reliably

distinguishing rival song from calls during the breeding season is therefore crucial. Here, we investigated how these two acoustic contexts influence male vocal behavior in the wild using playback of either male songs or ‘huit’ calls. In response to rival song, males engaged in counter-singing and regularly exhibited song matching behavior. In contrast, ‘huit’ call playback elicited longer songs with shorter inter-song gaps and the production of novel, high-pitched whistle-like syllables that were not observed in male-male interactions. These context-specific adjustments in temporal and spectral features indicate flexible categorization of socially relevant signals. To directly test categorical perception, we conducted a morphing experiment using playback stimuli that formed acoustic continua between song and call syllables. Males responded non-linearly along these continua, showing sharp behavioral transitions at distinct points rather than gradual changes. Responses partially clustered into male-directed or female-directed patterns, revealing clear perceptual boundaries. Together, these findings provide behavioral evidence that nightingales categorically perceive song and call types and tailor their vocal output accordingly.

PS2.80

Learning, memory and cognition

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Archerfish exhibit spatiotemporal exploratory behavior

Decision-making is fundamental to animals’ everyday behavior, enabling them to survive in constantly changing environments and achieve desired outcomes. Here, we investigate decision-making in archerfish, trained to select a target object over a non-target in a reinforcement-learning task. We aimed at understanding what happens after the animal becomes an expert in the task. Specifically, how it weighs the explorative versus the exploitative nature of its decision. Indeed, we found that all subjects learned to select the target object and achieved expert performance. However, after expert performance was reached, target selection became unstable. That is, longitudinal tracking revealed extended epochs in which subjects predominantly chose the non-target, followed by epochs in which they chose the target again, indicating exploration in time. Likewise, spatial analysis revealed similar exploratory behavior in the spatial domain. In addition, we asked whether choices were influenced by recent outcomes. We found that choice on a given trial was mostly independent of what has happened in the previous last few trials. Furthermore, during temporary reward omission, subjects maintained stable choice behavior and task engagement. Ultimately, this study shows that archerfish behavior is robust to sudden changes and explorative in nature, with broader implications on current understandings of decision-making within unpredictable environments. Notably, our findings differ from those reported in humans, rats, and mice, highlighting that exploratory behavior and decision-making vary substantially across vertebrate species.

PS2.64

Auditory systems and acoustic signaling (Part 2)

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Brain-wide hierarchical and sexually dimorphic tuning for social vocalizations

Acoustic communication is widespread among vertebrates and central to social behavior. Yet how brain-wide circuits identify conspecific signals and distinguish acoustic elements with different, often sex-specific social valence remains poorly understood. Here we present the first whole-brain analysis of neuronal responses to conspecific vocalisations in vertebrates, using the transparent fish *Danionella cerebrum*. Combining volumetric calcium imaging with playbacks probing the stimulus space of the natural sound repertoire, we uncover an unexpectedly early and specialized processing hierarchy: hindbrain nuclei already segregate vocalization-like pulse trains from tones, midbrain regions sharpen these representations and extract temporal features that define vocalization type, and the central posterior thalamic nucleus responds selectively to conspecific vocalization rates and thus acts as a gate for social sounds. Male and female brains share this early feature code but diverge in diencephalic and telencephalic regions, where identical acoustic features evoke sex-specific population activity patterns that parallel dimorphic behavior. Together, our results provide the first cellular-resolution, brain-wide account of social sound processing in a vertebrate, from early categorical segregation to thalamic gating and sex-specific population responses in social circuits.

PS1.38

Social behavior and neuromodulation (Part 1)

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Interactions between social isolation, serotonin, and behavior in different crayfish species

In most animals, serotonin (5-HT) is a major modulator of neurobehavioral activity, including along the gut-brain axis. However, how social isolation is linked to hormonal 5-HT and how this affects behavioral output remains poorly understood. We first performed electrochemical measurements of nanomolar 5-HT concentrations in the hemolymph of crayfish species from two different genera, *Procambarus clarkii* and Faxonius spp., after communal housing (COMs) or social isolation (ISOs). We found that COMs exhibited similar 5-HT levels, but *P. clarkii* experienced its highest 5-HT levels after 7 days of isolation while this was observed after 1 day of isolation in *F. spp*., possibly indicating different responses to isolation stress. Because 7-day ISOs were unfed in our first study, we also measured 5-HT levels in 7-day ISOs that were fed daily with shrimp pellets. Surprisingly, in *P. clarkii*, 5-HT levels in the hemolymph were lower in fed animals compared to unfed ones whereas in F. spp., 5-HT levels were higher after feeding. Because shrimp pellets contain tryptophan (TRP), a precursor of 5-HT, we speculated that these differences might be related to 5-HT synthesis in the crayfish gut and subsequent uptake into the hemolymph. Thus, we quantified place preference in a tank with light-dark chambers, a test of anxiety-like behavior, after feeding the animals a diet supplemented with TRP. In line with our 5-HT measurements, we found that *P. clarkii* showed greater avoidance of the brighter area after TRP diet compared to controls, whereas *F. spp*. exhibited the opposite behavior, nearly doubling their time spent in the light. In addition, *P. clarkii* crossed less frequently between dark-light chambers after TRP diet whereas *F. spp*. explored both sides more often. In summary, our data suggest that social isolation increases hormonal 5-HT levels in crayfish, but the temporal pattern differs between species, possibly due to differences in food supply and social environment in their natural habitats. Importantly, changes in hormonal 5-HT might be related to intestinal 5-HT biosynthesis since diet supplemented with TRP was effective in inducing species-specific behavioral changes.

PS3.79

Olfaction, taste and chemical sensing

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Localization of olfactory receptor co-expression in the *A. aegypti* antennae using HCR-FISH

Vector-borne diseases kill over 700,000 people per year, 90% of which are due to diseases spread by mosquitoes. Understanding the mechanisms of odor sensation and host seeking will allow us to develop more effective repellents and lessen the impact of these serious diseases. New findings have reported that the host-seeking mosquito *Aedes aegypti* exhibits a non-canonical olfactory system organization wherein olfactory sensory neurons can co-express more than one olfactory receptor, contrary to the traditional understanding of mammalian and insect olfactory system organization. Although single-nucleus RNA-sequencing (snRNA-seq) data has defined clusters of neurons in the mosquito antenna which co-express the same receptors, it is not known where on the antenna these co-expressed cells are located. In this work we use Hybridization Chain Reaction Fluorescence In Situ Hybridization (HCR-FISH) on the *A. aegypti* antenna to determine the spatial location of cells which co-express a subset of olfactory receptors with overlapping expression patterns. We find that there are distinct patterns of cells observable on the antenna whose receptor co-expression patterns correlate to clusters observed in snRNA-seq data. These expression patterns are consistent across antenna, suggesting a functional consequence of the specific receptor combinations we quantify in this work. We will determine the function of this co-expression by creating transgenic strains driving Genetically Encoded Calcium Indicators (GECI) in cells which co-express these receptors with the goal of performing functional assays on live mosquitoes. This work will highlight the relevance of co-expression in the *A. aegypti* antenna to odor coding in the mosquito brain and give us insights into how nervous system organization influences accurate representation of relevant environmental cues.

PS3.7

Motor systems, sensorimotor integration and behavior (Part 3)

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Striosome-matrix dissociation during distinct vocal behaviours in bats

Vocal communication requires flexible neural control integrating sensory, motor, and internal state information to produce context-appropriate vocal signals. Bats provide a powerful model to study such flexibility because they produce two functionally distinct vocalisation types: self-directed echolocation calls for spatial orientation and socially directed vocalisations for communication. However, the neural mechanisms enabling context-dependent switching between these vocal behaviours remain poorly understood. Here we mapped brain-wide expression of the immediate early gene Egr1 associated with context-dependent vocal behaviour in the bat *Carollia perspicillata* using in situ hybridisation. Adult males were recorded during echolocation, courtship behaviour, or quiet resting. Brain-wide Egr1 expression was quantified and analysed using complementary normalisation schemes to identify behaviour-related expression patterns. Both vocal behaviours induced widespread neural activation relative to quiet controls across cortical, limbic, and midbrain vocal-motor networks. Across analytical approaches, however, the striatum emerged as a robust discriminator of vocalisation context. Echolocation strongly increased Egr1 expression in both the matrix and striosomal compartments of the caudate nucleus, whereas courtship only elevated expression in the striosomes. These findings parallel social-context-dependent basal ganglia dynamics described in songbirds. In line with known mammalian basal ganglia circuitry, we propose a striosome-substantia nigra pars compacta-matrix gating framework, in which striosomes may influence striatal dopaminergic tone and thereby bias matrix-mediated sensorimotor processing according to behavioural context, consistent with prior evidence that dopamine modulates echolocation call structure. Together, our findings highlight compartment-specific basal ganglia dynamics as a potential neural mechanism for context-dependent vocal control in bats.

PS1.91

Evolution and development

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Predicting brain anatomy from cranial endocasts in snakes

Mapping the evolution of brain anatomy can provide a window into the ecology and behaviour of extinct animals, but it requires the characterization of brain anatomy across many species, frequently including those that exist only as fossils. Since vertebrate brains are encased in bone, the cranium, we use the interior shape and size of the cranium (referred to as an 'endocast'), including from fossils, as a proxy for the shape and size of the brain. However, the extent to which the shape of the brain is imprinted on the cranium, and the variation in this relationship between clades, are rarely quantitatively considered. We developed a dataset of brain-endocast pairs for the same specimens across extant snakes as a model group to test the brain-endocast relationship. Snakes have a complete cranium, like mammals and birds, but evolved it independently from species with incomplete crania, providing a parallel system in which to test the fidelity of brain-cranium relationships. We used traditional microCT to scan the skull of each specimen, from which we digitally 'filled' the cranium to create endocasts. We then stained each specimen with buffered Lugol's iodine and microCT scanned it again to image the specimen's soft tissue (a technique known as diceCT). From the soft-tissue-CT we digitally extracted the brain of each specimen. We then examined brain-endocast volume and shape relationships and compared the variation in brain-endocast fidelity across brain regions and species. We demonstrate that brain shape and size can be predicted from endocasts, but with caveats that not all brain regions are equally well represented by an endocast. We will discuss which brain regions are most and least reliably represented and what the implications are for predicting ecology and behaviour from endocasts.

PS3.65

Vision and photoreception (Part 2)

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Visual behavior and functional regeneration in the central nervous system of the axolotl

The salamander axolotl (*Ambystoma mexicanum*) can regenerate parts of its central nervous system after injury. Regenerated neurons show both activity and connectivity, yet the functional restoration of complex circuitry and behavior remains largely unknown. This is in part due to the lack of standardized behavioral assays to study the function of neuronal circuits. Moreover, little is known about the natural behavioral repertoire of the axolotl.

Here, we developed a setup to study visually guided behaviors in the axolotl and characterized two ethologically relevant behaviors: prey capture and phototactic light preference. For this, we use a projection system with stimuli being presented on a screen above the axolotl while recording the animal from below. The versatility of this setup will allow for adaptations to study a wider range of natural behaviors, such as visual avoidance and escape responses.

In the established paradigms, we observed a prey capture-like snapping response toward small dot stimuli, but interestingly could not observe a preference for light or dark environments as observed in many other aquatic species.

Next, we used the setup to study the functional recovery of vision after injury. Optic nerve transection resulted in initial ablation of prey-capture behavior which was restored following regeneration, indicating a successful restoration of the visual circuit.

PS3.44

Social behavior and neuromodulation (Part 2)

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Seasonal tissue-specific transcriptomes in seasonally singing canaries

Seasonal changes in vocal behaviors of songbirds provide a model for understanding how internal states regulate socially-driven behavior. In canaries (*Serinus canaria*), the transition from the non-breeding to the breeding season brings pronounced changes in song pattern and song behavior, closely linked to rising blood testosterone levels. Here we investigate the coordinated transcriptional responses across brain areas and peripheral tissues to the systemic testosterone signal in parallel with changes in singing behavior. We combined behavioral analysis in a semi-natural environment, endocrine profiling, and multi-organ transcriptomics to investigate this seasonal regulation. Specifically, bulk RNA sequencing was performed across multiple peripheral tissues (liver, heart, spleen, intestine, syrinx muscle, kidney, gonad, pectoral muscle) and brain regions (hyperpallium, cerebellum, preoptic area) as well as spatial transcriptomics of brain regions including song controlling brain areas. Singing differed seasonally concerning the song pattern and the singing performance such as the body posture. Seasonal transcriptional changes were tissue-specific, with the gonads showing massive remodeling with about 4,500 differentially expressed genes (DEGs), whereas the liver and heart exhibited only tens of DEGs and all other investigated organs showed, surprisingly, minimal changes despite the presence of androgen receptor (AR), the receptor for testosterone and a known transcription factor.

While AR mRNA expression (as verified by RNAscope in-situ hybridization) remained largely stable across most tissues, it was specifically reduced in breeding gonads, suggesting a localized negative feedback mechanism. The heart maintained stable AR mRNA levels but upregulated SRD5A1 mRNA, coding for 5?-reductase, an enzyme converting testosterone into the more potent AR ligand dihydrotestosterone. In the brain, bulk transcriptomics showed 11 DEGs in the hyperpallium, 5 DEGs in the cerebellum, and zero DEGs in the preoptic area. These results will be further detailed by using spatial transcriptomics of POA, HVC and RA regions. These results collectively suggest that seasonal changes in testosterone affect the brain as well as certain peripheral organs in tissue- and area-specific ways.

PS2.2

Motor systems, sensorimotor integration and behavior (Part 2)

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Haltere modulation of the visual system for rapid flight turns in *Drosophila melanogaster*

Animals must distinguish external perturbation from self-generated motion to prevent equilibrium reflexes from negating voluntary movements. In *Drosophila melanogaster*, visual interneurons sensitive to wide-field optic-flow receive an inhibitory pre-motor signal during rapid flight turns termed saccades. However, the source of this signal remains unclear. In flies, vestigial hindwings known as halteres detect changes in angular velocity. Beyond acting as passive gyroscopes, descending visual input perturbs the halteres to induce compensatory reflexes facilitating active maneuvers. Halteres may also drive neck muscles to shift gaze during saccades. Here we investigate how halteres modulate optomotor reflexes for saccades through interneurons that transform excitatory haltere feedback into inhibitory output to the visual system. Connectomics analyses reveal a population of interneurons, known as crab lobula plate tangential cell recurrent neurons (cLPTCrns), that may link haltere feedback to inhibition of the wide-field optic-flow sensitive lobula plate tangential cells (LPTCs) suppressed in active turns. We characterized synaptic connectivity from haltere afferents to cLPTCrns using the recently published brain and nerve cord (BANC) connectome. We then optogenetically altered cLPTCrn activity in a magnetic tether that preserves haltere feedback while measuring optomotor reflexes. Activating cLPTCrns results in shorter saccades and exacerbates compensatory head yaw trajectory, nearly doubling the peak head yaw velocity for saccades. Ongoing work is examining the effects of silencing cLPTCrns on saccade dynamics. Our findings demonstrate cLPTCrns help modulate optomotor responses for rapid flight turns, enhancing our understanding of how haltere feedback co-opts reflex circuitry in the visual system. This suggests a novel mechanism for detecting and facilitating self-motion.

PS1.2

Motor systems, sensorimotor integration and behavior (Part 1)

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Why would flies play on a carousel?
Play-like behaviour, whether social or individual, is described across the animal kingdom, with a clear emphasis on vertebrate species. Invertebrates also display play-like behaviour, most of which has been reported in a social context and in eusocial insects. The neurobiological basis of self-directed play-like behaviour remains elusive. To address this void, we examined exploratory behaviour in the vinegar fly *Drosophila melanogaster* by presenting single males with an enriched environment encompassing voluntary access to a spinning platform - a carousel. We demonstrate that flies exhibit complex interaction with a moving carousel with spontaneous avoidance, but also repeated and prolonged visits. These interactions were idiosyncratic and conserved across days in individual animals. Moreover, when presented with two alternatingly spinning platforms, individual wildtype flies repeatedly sought the spinning carousel - this spatial alternation is consistent with a positive valuation of the experience. By being voluntary/intentional, stress-free, repeated, non-ethotypical, and not immediately relevant for survival, we conclude that the observed sensation seeking on the spinning carousel fulfils the key criteria for play-like behaviour defined in the field. Importantly, we can now probe the adaptative value of voluntary passive movement play-like behaviour. We argue that self-exposure to centripetal force represents intentional exafferent stimulation, which provides an efficient way to improve self-perception and hence motor control. This work therefore opens the possibility of studying in detail both the behavioural consequences of play-like behaviour and their neuronal implementation.

PS3.57
Vision and photoreception (Part 2)
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Why did the fly cross the gap? - A computational model of distance tuning in neural superposition compound eyes
Neural superposition in fly compound eyes enhances light sensitivity by pooling signals from photoreceptors that sample the same region of visual space. However, the optical axes of photoreceptors projecting to a single lamina cartridge are not perfectly parallel, but instead converge at a point a few millimeters in front of the eye. The exact convergence distance varies both between and within species. At short viewing distances, this misalignment leads to distance-dependent differences in receptive field overlap. The range over which these differences become significant corresponds to behaviorally relevant distances, such as those involved in gap crossing and courtship. We therefore explored whether flies could sense depth within this `personal space' purely from the geometry of neural superposition. To this end, we combined a computational model of the fly eye with a disparity-tuned neuron model originally developed for stereoscopic prey capture in praying mantises, and simulated the responses of a lamina cell to a moving stimuli at different distances. This approach is motivated by an analogy to light-field cameras, which also sample light from multiple directions and use the disparities between these samples to digitally refocus images and estimate depth. Across variations in stimulus direction, movement axis, and lamina model, we found that lamina neuron responses contain a distance-dependent component whose expression depends on both stimulus parameters and the chosen model. Notably, analysis of the early response dynamics revealed that the initial response gradient (within the first 10\,ms) is maximal at the convergence distance of photoreceptor optical axes. We further show that changes in eye size systematically shift the convergence distance, consistent with anatomical observations that flies with larger eyes have smaller interommatidial angles. Our simulations produced shifts in the lamina response corresponding to shifted convergence distances.

Our results point towards a geometry-derived component in the response of lamina cells that is specific to object distance and invariant to other stimulus properties. This may be interpreted as neural superposition functioning analogously to a light-field system that is effectively focused on a behaviorally relevant distance.

PS3.96

Methods

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Osteological correlates of hearing abilities in birds and their application to extinct species.

Sensory abilities dictate how animals interact with one another and the environment and are therefore key to all behaviours. The acuity and sensitivity of a sensory modality are often assessed via behavioural and physiological testing, but are also reflected in the anatomy of sensory organs and brain regions processing sensory information. For example, increased acuity is typically associated with more sensory receptors and larger processing brain regions. In birds, this is exemplified by owls, which have elongated basilar papillae and expanded auditory regions that support a broader hearing range and precise localization of sounds. Although behaviour, physiological, and histological techniques can be used on extant species, inferring the sensory abilities of extinct species relies on osteological traits. In the auditory system, the length of the endosseous cochlear duct (ECD), the bony housing of the basilar papilla, is positively correlated with best hearing frequency and hearing range across archosaurs. This equation has been used to estimate hearing abilities of dinosaurs and other extinct species, but some of the estimates generated are questionable. Using audiogram data and CT scans of 30+ species, we analyzed ECD length and tympanum size in relation to best hearing frequency and frequency range. We show that tympanum size is not significantly correlated with audiogram data. Relative, but not absolute, ECD length is positively correlated with best hearing frequency and frequency range, but with a different slope than the previous analyses. We then calculate revised estimates of the hearing abilities of several extinct species, but emphasize that the presence of several outliers suggests that ECD length alone is not always sufficient to estimate hearing abilities. We therefore urge caution in the application of this method to dinosaurs or early birds and emphasize the need for more audiogram and anatomical data.

PS2.20

Motor systems, sensorimotor integration and behavior (Part 2)

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Cooperative robustness to warming in the stomatogastric system of the crab *Cancer borealis*

Understanding how warming affects animal physiology is increasingly important as ocean temperatures rise at unprecedented rates. We study the stomatogastric nervous system of the crab *Cancer borealis*, including the pylorus involved in food filtering. In this system, muscle contraction depends on several temperature-sensitive levels of organization, from neuronal activity to neuromuscular transmission and contractile machinery of the muscle fibers. Because temperature affects each of these processes differently, maintaining the stereotyped neural and muscular activity required for digestion is a major challenge. Previous work showed that pyloric neurons increase their firing frequency with temperature until the rhythm crashes at extreme values. Here, we ask how interactions across these levels of organization cooperate to maintain stereotyped activity across the temperature range. Simultaneous recordings of nerve activity, muscle fiber membrane potential changes (excitatory junctional potentials, EJPs), and muscle contraction show that as temperature rises, nerve activity and EJPs are maintained, whereas contraction fails first. When muscles are stimulated at a fixed low frequency, mimicking cold neuronal activity, contraction decreases with warming. Increasing stimulation frequency to mimic hot neuronal activity partially rescues contraction. Thus, the spontaneous temperature-dependent increase in neuronal firing frequency provides partial compensation, but this mechanism ultimately fails at higher temperatures. We further show that warming hyperpolarizes muscle fibers, likely through activation of two-pore potassium channels, moving the membrane potential farther from the contraction threshold. However, high potassium contractures demonstrate that the contractile machinery is still functional at high temperatures. Together, these results show that multiple mechanisms cooperate across levels of organization to preserve function over a broad temperature range.

PS1.40

Social behavior and neuromodulation (Part 1)

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Neural and nutritional mechanisms regulating division of labor in ant alloparental care

Feeding and parental caregiving are essential behaviors across diverse animal species. Parental care requires a shift in priority from self-directed feeding, to provisioning and nurturing offspring. This shift may have evolved via evolutionary modifications of ancestral neural circuits originally dedicated to feeding behaviors, laying the groundwork for complex social interactions. In clonal raider ant colonies (*Ooceraea biroi*), nutritional state biases the division of labor during parental care: starved ants perform brood care, while well-fed ants forage. Additionally, neuropeptides that regulate feeding in *Drosophila* also modulate ant caregiving behavior. This suggests that ancient feeding circuits have been repurposed to facilitate active parental care in ants. To test this hypothesis in *O. biroi*, I am leveraging novel functional tools in this species, including in vivo calcium imaging and an emerging whole-brain connectome. I will manipulate nutritional state to determine its effect on caregiving behavior and differential gene expression in the subesophageal zone (SEZ), a brain region that primarily regulates feeding behavior in flies. I will also characterize synaptic connectivity and in vivo sensory representations in the ant SEZ, to understand how nutritional and social cues shape parental care behaviors.

PS1.72

Spatial orientation and navigation (Part 1)

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Caching in a shell-dwelling cichlid

A remarkable behavior exhibited by many vertebrates is caching, which involves hiding resources from intra- or interspecific competitors to retrieve them later. Resources may be cached for several weeks or even multiple months, spanning a significant proportion of an individual's lifetime. Furthermore, resources can be stored across multiple sites. Remembering multiple locations over an extended period is a cognitively demanding task. Although extensive research has been conducted on caching in birds and mammals, comparable studies in teleost fish are lacking. The shell-dwelling cichlid *Lamprologus ocellatus* (LO) inhabits sandy substrates in the shallow waters of Lake Tanganyika. LO are obligate shell dwellers whose life cycle revolves around abandoned snail shells that individuals aggressively defend against intruders. Males establish harems consisting of three to four females, with each individual occupying and maintaining its own shell. Given the critical importance of shells for the life cycle of LO, possessing multiple shells is advantageous. Excess shells within a territory are frequently blocked or buried, potentially preventing competitors from accessing them. We demonstrate, through laboratory experiments and field observations, that LO retrieve previously buried shells. Using activity-dependent and cell type-specific markers, we identified neural correlates of this behavior in the dorsolateral pallium and subpallial areas. Our findings provide evidence for caching behavior in a teleost species and contribute to understanding the evolution of spatial cognition across vertebrates.

PS2.11

Motor systems, sensorimotor integration and behavior (Part 2)

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Characterizing a larval feeding paradigm in the sea urchin, *Lytechinus pictus*

Many marine zooplankton use cilia for locomotion, and often, for feeding. However, the neuronal circuit behind the complex coordination required by ciliary feeding behavior has not been identified. To gain insights into this ecologically important behavior, we must first have a robust behavioral assay and genetic tools in a species that relies on cilia for feeding. The genetically-enabled sea urchin, *Lytechinus pictus*, provides a unique opportunity to not only spatially correlate neuronal populations with behavior, but also to test the hypothesized circuit through functional calcium imaging and loss of function experiments. However, before this behavior can be studied functionally, ciliary feeding must first be adapted to a quantifiable assay amenable to automated analysis. This study establishes a controlled and replicable protocol and arena to expose sea urchin larvae to food stimuli, and record the behavioral response. Using Social LEAP Estimates Animal Poses (SLEAP) to track particle movement, particle collection, muscular movement of the mouth, and esophageal contraction, feeding behavior can be quantified and compared across treatments. This platform is currently being used to characterize knockout phenotypes and will be paired with pan-neuronal calcium imaging to uncover the molecular regulators of ciliary feeding. This work establishes a behavioral paradigm in the larval sea urchin,

enabling research into how a non-cephalized nervous system coordinates complex feeding behavior.

PS3.33

Social behavior and neuromodulation (Part 2)

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Impact of social isolation on the neural responses of a wasp with identity recognition

Polistes fuscatus wasps possess individually distinctive color patterns on their face, which they can use to visually recognize and discriminate each other. The development of this ability is dependent upon social experience. Previously, we found a localized physiological population of neurons, we refer to as wasp cells, in *Polistes fuscatus* that are selective to forward-facing wasp images in the lateral protocerebrum. Additionally, the synaptic volumes of high-order visual centers in these same brain areas are differentially impacted by social experience. We now perform multichannel extracellular electrophysiology recordings in the brains of *P. fuscatus* wasps that have been isolated for their entire adult lives and have never seen another adult wasp. We find a striking difference in the relationship between the selectivity for forward-facing wasp images with and without color across the brains of these animals. In normally reared animals there is a very tight relationship between a unit's selectivity for wasp shape with and without wasp internal features present. Here in isolated animals this relationship is almost entirely gone. However, we do still find units that are selective for forward facing wasp images with and without internal wasp features. This suggests that there is a developmental program in *Polistes fuscatus* that produces wasp cells in appropriate regions of the brain and experience is not necessary for social image selectivity to develop in these circuits. However, the proportion of selective units and their degree of selectivity to different wasp features is impacted by social experience. This finding suggests that connectivity between circuits responding to wasp shape and wasp color features may be what is altered by social experience, and ultimately gives rise to the different social discrimination abilities between socially reared and socially isolated animals.

PS2.102

Metabolism, biological rhythms and homeostasis

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Interaction between day length and food availability in driving degeneration of singing behavior

Individuals in temperate regions face significant annual changes in temperature, photoperiod, and resource availability that often give rise to season-specific traits. The flexibility of these traits allows organisms to adjust to local environmental conditions and produce appropriately contextualized behavior. To examine how environmental sensing ultimately drives neural and behavioral plasticity, we tested the interaction between light duration and the timing of food availability in the songbird, Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*). White-crowned sparrows are migratory

songbirds that demonstrates remarkable neurophysiological plasticity in their song control circuit and resulting singing behavior. Each spring during northern migration, the sensorimotor song control nucleus, called HVC nearly doubles in neuron number within the male brain. This growth, in addition to the growth of downstream target nuclei, support the singing of stereotyped breeding-condition song. As day length decreases (and local temperatures and food availability decrease), HVC degenerates and sparrows discontinue singing before migrating southward to their wintering grounds. To test the interaction between food availability and photoperiod in driving non-breeding season associated behavior, we restricted the timing of feeding to that of a short-day photoperiod in sparrows housed in both short- and long-day photoperiods. We found that food timing, independent of light, is sufficient to drive a cessation of singing behavior and now we are examining whether this is driven by underlying degeneration in HVC. Better understanding the relationship between photoperiod and resource availability will provide further insight into how environmental cues shape neurophysiology and how populations may change as photoperiod and climate become increasingly mismatched.

PS1.29

Social behavior and neuromodulation (Part 1)

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Digital avatars reveal neural dynamics of social behaviour in wild cichlids

Understanding how the brain generates social behaviour remains a challenge because social interactions involve continuous feedback between sensory input and behavioural output. In addition, captive versus wild conditions can fundamentally alter social responses. Captive animals may experience simplified social environments that alter natural behaviour, whereas studies of wild social interactions occur under uncontrolled conditions that confound interpretation. Here we measured brain activity during social behaviour using standardized digital playbacks displayed to wild shell-dwelling cichlids (*Lamprologus ocellatus*) on the shores of Lake Tanganyika. We captured focal animals from natural social environments and exposed them to 3D avatars of heterospecifics, conspecifics, and a control sphere, each programmed with identical kinematic behaviour. We tracked and scored the responses of focal animals, which recognized the different stimuli and displayed graded behavioural responses: spheres elicited minimal aggression or approach behaviour, heterospecifics elicited intermediate aggression and limited approach, and conspecifics elicited strong approach and territorial responses including biting and ramming. We then collected brains in the field and transported them to the laboratory to quantify pS6 activity across nodes of the social decision-making network (SDMN). Digital social input reorganized coordination across the network rather than altering overall activation: sphere trials produced highly synchronized activity across regions, heterospecific encounters reduced global coordination, and conspecific encounters generated the most modular and segregated networks. Increasing social salience thus shifted the brain from highly correlated to differentiated states. Behavioural output was also associated with changes in activity: individuals varied in their responses to conspecific stimuli, and aggression was positively associated with activity in the medial preoptic area. By bridging experimental control of social stimuli with behaviour expressed in the wild, these results reveal how SDMN activity integrates social input and behavioural responses.

PS1.31

Social behavior and neuromodulation (Part 1)

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Using poison frog to explore how repeated early life experiences shape the nervous system

Aggression towards peers is common during early life in many taxa, with important consequences for immediate survival and life-long success. However, our understanding of the underlying mechanisms of juvenile aggressive behavior and how they are shaped by early life experiences is very limited. We are using dyeing poison frog (*Dendrobates tinctorius*) tadpoles - which attack, kill and cannibalize other tadpoles - to understand the neural and genomic basis of aggressive behavior. In particular, we tested how repeated experiences of aggression affect an individual's development and behavior as well as how these experiences get embedded into the nervous system in the long-term. To test this, we tracked poison frog juveniles throughout development, regularly exposing them to encounter assays in which they freely interacted with other tadpoles. We determined aggressive and submissive individuals from these encounters and tested for differences in growth rate, hiding and aggressive behavior. We collected brains of all individuals to identify how behavior is mediated in the moment (e.g. gene expression) and how experiences shape the nervous system long-term (e.g. epigenetics changes). We expect to find submissive individuals to show a reduced growth rate, increased hiding and decreased aggressive behavior compared to aggressors. We expect that these differences are reflected in neural activity and gene expression. Our study contributes to our understanding of juvenile aggression and sheds light on how early life experiences shape the nervous system and how these experiences get retained long-term.

PS2.63

Auditory systems and acoustic signaling (Part 2)

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Multimodal reference brain atlas of adult *Danionella cerebrum*

Mechanistic accounts of brain function require a common coordinate system in which structural, molecular and functional data can be integrated and compared across individuals. The teleost genus *Danionella* is unique among vertebrates in retaining lifelong transparency, allowing non-invasive, cellular-resolution functional imaging across the entire adult brain. A reference atlas in this model would therefore provide a strong foundation for causal and comparative circuit studies. Here we present an integrated anatomical, molecular and functional reference brain for adult *Danionella cerebrum* as a standardised atlas resource. Using a transgenic nuclear fluorescence marker, whole-mount tissue clearing and high-resolution two-photon microscopy, we generated an average reference brain from 21 adult fish to create a common coordinate system. Whole-mount in situ hybridisation for 29 neuronal markers, complemented by tract annotation from structural imaging and tracer injections, enabled us to segment 203 neuroanatomical regions. We found pronounced sex differences in telencephalic, cerebellar and hindbrain nuclei, revealing sexually dimorphic organisation across multiple brain regions. All data and segmentations are made openly accessible, providing a community resource for studies of circuit function, molecular makeup and sexual dimorphism in an optically accessible adult vertebrate brain.

PS3.88

Electrosensory systems

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Multiple electrosensory maps in the glass catfish hindbrain

What neural circuit changes underlie the evolution of active sensing? Electric fish possess electric organs that generate electric fields around the body, and specialized electroreceptors that detect distortions in these fields and communication signals from nearby fish. In addition to this active system, they retain a passive electrosensory system that detects weak electric signals produced by prey. Sensory inputs from these receptors are transmitted via the lateral line nerves to the electrosensory lateral line lobe (ELL) in the hindbrain, where they are organized into topographic maps that correspond to parallel processing streams. However, it is still unknown how such complex circuitry in an active electric sensing system has evolved. Catfish offer a unique opportunity to explore this mystery because they possess only a passive electrosensory system, yet share a common ostariophysan ancestry with gymnotiform electric fishes. Although the overall topographic layout and primary afferent pathways are largely conserved in catfish, the detailed processing mechanisms remain unclear. To address this question, we used glass catfish (*Kryptopterus vitreolus*). Anterograde labeling of the electrosensory afferents revealed that primary electrosensory neurons project to the ELL in a topographic manner. To further characterize these projections, we combined neural tracing with tissue clearing to reconstruct them in 3D, revealing that the electrosensory neurons may have multiple targets within the ELL. These findings suggest that catfish possess parallel processing mechanisms in their passive electrosensory system, similar to those in the active electrosensory system of electric fish.

PS3.28

Social behavior and neuromodulation (Part 2)

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Neuromodulation of temperature-dependent behaviors in *Aphaenogaster* ants

Animals are often adapting to their external environments, and as temperatures rise with climate change these adaptations may become more crucial for survival. The neurotransmitter dopamine is sensitive to temperature changes and supports behavioral adaptations to high temperatures. However, it is not known whether variation in dopaminergic signaling corresponds to species differences in behavioral responses to high temperatures. We tested the hypothesis that larger shifts in behavior with thermal stress are associated with reduced dopaminergic signaling in two closely related species that live in the same environment, *Aphaenogaster tennesseensis* and *Aphaenogaster picea*. Here we show that *A. picea* has significantly more dopamine neurons, while *A. tennesseensis* exhibits more temperature sensitivity. While *A. tennesseensis* typically displays more aggressive and exploratory behaviors than *A. picea*, high temperature increases these behaviors in *A. tennesseensis* but not *A. picea*. *A. tennesseensis* also has higher mortality at elevated temperatures. Pharmacologically manipulating dopamine during behavioral

observations clarifies the role of dopamine in mediating temperature-dependent behavioral responses in both *Aphaenogaster* species. Our work suggests that variation in dopaminergic signaling may contribute to species differences in behavioral responses to thermal stress.

PS1.69

Spatial orientation and navigation (Part 1)

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Sensory-guided action planning in echolocating bats during free-flight navigation

Understanding how animals select and plan voluntary actions in dynamic environments remains a fundamental challenge in systems neuroscience. Although recent technological advances have provided insight into external-cue conditioned actions, our understanding of how an animal can select complex, self-initiated actions among infinite action possibilities in a dynamic environment is still limited. To address this challenge, we study action planning in echolocating big brown bats (*Eptesicus fuscus*), which produce frequency-modulated sonar calls and adjust the directional aim and timing of their sonar calls in response to echo information they have processed and the information they are seeking. This quantifiable metric of spatial gaze, combining the temporally separated neural signals representing perception and action, therefore provides an empirical window to probe sensory-guided action selection. We developed a navigational task in which bats must locate a goal from outside a large maze and navigate inside the maze based solely on previously acquired information, without real-time sensory access to the location of the goal. To quantify behavior, we use high-speed infrared cameras to track three-dimensional flight trajectories, and 360-degree high-fidelity microphone arrays to characterize sonar beam features, including direction, frequency, and intensity. Results demonstrate that bats successfully learn to navigate to the goal. Analysis of echolocation call patterns revealed increased call rates prior to landing, consistent with previously reported findings and confirming behavioral signatures of action planning. Future analysis will include the directional aim of echolocation corresponding to the bat's flight trajectory and navigational decisions. These behavioral findings will then be linked to neural substrates through multichannel electrophysiological recordings in the hippocampus during free-flight 3-D navigation.

PS2.4

Motor systems, sensorimotor integration and behavior (Part 2)

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The neural mechanisms underlying the evolution of flower-visiting behavior in *Drosophila*

Animal visitation of flowers plays a key role in animal-plant interactions. However, the neural mechanisms underlying the evolution of flower-visiting behavior remain largely unexplored. To address this question, we focused on *Drosophila elegans*, a species that evolved flower-visiting behavior from non-flower-visiting ancestors approximately 15 million years ago. In

the field, *D. elegans* visits blue *Ipomoea* flowers for foraging and reproduction. To investigate how *D. elegans* recognizes the host flowers, we first measured landing responses when flies were presented with blue, green, beige, and yellow disks of equal subjective brightness. The flies selectively landed on the blue disk, which resemble their host flowers, indicating a reliance on color cues for flower recognition. Furthermore, mutants with impaired R7 and blue-sensitive R8 photoreceptors, which contribute to color vision, failed to visit the blue disks. This indicates that *D. elegans* depends on color information transmitted via these inner photoreceptors. To explore how the visual system of *D. elegans* may have adapted for flower recognition, we compared it with that of the non-flower-visiting relative, *D. melanogaster*. The rhabdomeres of the blue- or green- sensitive R8 photoreceptors were thicker in *D. elegans*, suggesting enhanced sensitivity to these wavelengths. Consistently, electroretinogram recordings revealed approximately 1.5-fold higher sensitivity to blue light in *D. elegans*, suggesting a highly sensitive system for detecting host flower colors. In the downstream optic lobe, the relative size of the medulla, a major color-processing area, was larger in *D. elegans* relative to the central brain than in *D. melanogaster*. The anatomical feature implies that *D. elegans* allocates greater neural investment to color processing. Together, these findings suggest that functional adaptations in color-processing pathways contributed to the evolution of flower-visiting behavior in *Drosophila*.

PS2.44

Vision and photoreception (Part 1)

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Glowing and seeing red in deep-sea dragonfish (Stomiiformes)

The ocean's twilight zone (200-1000 m depth) presents big contrast to terrestrial habitat. One of the main differences is the scarcity of light reaching 0.1% of ambient light. The second source of light is bioluminescence, produced by the oceanic invertebrates as well as vertebrates. Since ambient light is depleted towards blue-green wavelengths (480-570 nm) with increasing depth, the animals are also primarily adapted to perceive predominantly in this wavelength and to emit in this blue-green light spectrum. Dragonfishes (Stomiidae) are the one striking exception, being able to operate in red to far-red wavelengths (around 650 nm) on top of the conventional blue-green. The complex scheme of luciferin functioning as a substrate for the enzyme luciferase, and the catalysis resulting in emission of light, is currently understood for only a slight portion of bioluminescent species. The chemistry itself is held in specialized glandular organs (photophores) and the red emission is obtained by a combination of different filters, reflective layers, and by transmission to a fluorescein (BRET) shifting the final emission to longer wavelengths. In this project, we are on the hunt for candidate gene(s) coding for luciferase enzyme, or its cascade. By comparative transcriptomics, we were able to pinpoint a narrow group of candidate genes. At first, we sampled 10 individuals from 4 species covering phylogeny of Stomiiformes (both red-glowing and blue-glowing species). By differential gene expression analysis of different types of photophores, we identified transcripts specific for photophores, and we complement those by the functional annotation. We believe that products of those candidate genes can be linked to bioluminescent machinery and might be involved in light

emission. Our candidates serve for subsequent functional tests and AI predictions. Here we aim to identify a candidate genes potentially coding for stomiid luciferase enzyme. The ability of some stomiids to operate in the red channel represents substantial evolutionary advantage, and we would like to see how those genes have evolved, including also other molecular context, such as how vision in red operates.

PS2.76

Spatial orientation and navigation (Part 2)

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What if the lab could go to the animal?

How do animals make decisions? This question lies at the heart of neuroethology, yet much of what we know comes from behaviors studied under constrained laboratory conditions. While these approaches offer control, they often come at the cost of context, particularly for behaviors such as navigation, migration, and collective motion, which are shaped by the environments in which they unfold. Bridging this gap requires not only recreating aspects of the natural world, but accessing behavior as it actively occurs within it. Here we present MATREX, an open-source, modular virtual reality platform designed to bring experimental control into ongoing natural behavior. Built from off-the-shelf components and 3D-printed modules, each unit delivers near-complete panoramic visual input to walking and flying animals and can operate both in the laboratory and off-grid in the field. This design enables controlled perturbations without removing organisms from their ecological or internal state. Using this system, we access behavioral states that are typically transient and difficult to reproduce. Death's-head hawkmoths, captured mid-migration, were studied in closed-loop flight for over hundred hours while navigating real world terrains and night skies along their migratory routes. Mormon crickets, collected from active field swarms, were probed in virtual bands to decode the interactions governing collective motion. In weaver ants, scrambling naturalistic scenes disrupts homing, revealing how navigation depends on structured visual memory and how responses differ between familiar and unfamiliar environments. In locusts, multiple arenas were networked to create shared virtual spaces in which individuals interact across distributed systems, linking moment-to-moment decisions to emergent swarm-level organization. Across these systems, behavior is not induced but encountered, stabilized, and gently perturbed without breaking its continuity. This allows decision-making to be studied as it is expressed, shaped by both internal state and environmental structure. By uniting ecological realism with experimental precision, MATREX offers a path toward understanding how cognition operates in the real world and how individual decisions scale into collective behavior.

PS3.72

Olfaction, taste and chemical sensing

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Transforming sense of scent: morphological and behavioural adaptation in sea snake tongues

Tongue flicking is one of the most prominent behaviours in snakes, used to bring chemicals from their environment to the vomeronasal organ to perceive their surroundings and gain information on prey or mates. The upward and downward movement of the tongue in air (tongue-flicking) is thought to attract odourants while the bifurcated tongue enables tropotaxis towards chemical gradients. In water, however, chemical cues are often more dispersed and ephemeral. It is unclear how the tongue has adapted during the transition from land to sea in snakes, and how much functionality was either lost, elaborated or compensated by other sensory pathways. In this study, we investigated how tongue morphology and ultrastructure relate to tongue-flicking in sea snakes, which are obligate marine reptiles, compared to their closely related terrestrial relatives (Hydrophiinae). To record prey-associated tongue flicks of terrestrial (*Acanthophis antarcticus* and *Notechis scutatus*) and sea snakes (*Hydrophis spp*.), captive snakes were exposed in behavioural experiments to a chemical cue versus a control on different sides of an enclosure. The number and duration of tongue flicks, side choices and kinematics of tongue flicks were analysed. We found differences in tongue-flicking behaviour that reflect anatomical differences in tine length, degree of bifurcation and surface structures between land and sea snakes.

PS2.23

Vision and photoreception (Part 1)

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Morphological variations of Kenyon cells in the mushroom body of swallowtail butterflies

The mushroom body (MB), a center for multimodal sensory processing in the insect brain, consists of the calyces (CA), peduncle, and lobes, which correspond to the dendrites, axonal fibers, and terminals of the intrinsic neurons, the Kenyon cells, respectively. We previously demonstrated that in the swallowtail butterfly *Papilio xuthus*, olfactory inputs innervate the outer zone of the CA, whereas visual inputs occupy the remaining areas, the inner zone and the rim of the outer zone of the CA. The spheroidal lobe of the *Papilio* MB consists of the ?/?, ?í/?í, and ? lobes, as described in other insects. To clarify how visual and olfactory information are conveyed within the MB, we examined the morphology of individual Kenyon cells using intracellular dye injection. We identified two types of unimodal Kenyon cells forming parallel pathways within the MB. Visual Kenyon cells extend their dendrites into the inner zone of the CA, and project their axons to the ventral area of the lobe, the ?/? lobe. In contrast, olfactory Kenyon cells arborize in the outer zone of the CA and send axonal terminals to the intermediate area of the lobe, the ?í/?í lobe. In addition, we identified Kenyon cells that extend their dendrites to both visual and olfactory input zones in the CA. These cells project to both ?/? and ?í/?í lobes. These anatomical findings provide a structural basis for the integration of visual and olfactory information in the MB, and suggest that such multimodal Kenyon cells may contribute to behavioral phenomena such as innate color preferences modulated by plant scent in foraging *Papilio* butterflies.

PS2.86

Learning, memory and cognition

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Habituation appears to involve distinct interstimulus interval-sensitive processes
Habituation is a form of learning that occurs when an organism decreases its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. The time between stimulus presentations - the interstimulus interval or ISI - greatly impacts the rate, depth, and memory of habituation. Animals habituate slowly and to a lesser extent at long ISIs, but the response decrement persists for longer than when the decrement is induced by short ISIs. Thus, at longer ISIs habituation learning is slower, but memory of this learning lasts longer than at shorter ISIs. To investigate whether this is caused by different habituation processes acting at short (10s) and long (60s) ISIs, we used our Multi-Worm Tracker to simultaneously monitor the behavior of hundreds of *Caenorhabditis elegans*, on Petri plates that receive mechanical taps at different ISIs. Worm's naive response to a tap is to reverse, moving backwards briefly. We tested worms with mutations in candidate genes at short and long ISIs and found mutants with altered habituation at one ISI. For example, animals with mutations in cmk-1, homolog of Ca2+/calmodulin-dependent kinases 1/4 and ogt-1, homolog of O-GlcNAc transferase have a habituation phenotype comparable to wildtype at short ISIs but have a clear alteration in habituation at longer ISIs. In addition, cmk-1 mutants are particularly sensitive to ISI and their habituation changes dramatically when the ISI is changed from 10s to 20s partway through an experiment. cmk-1 mutants habituation is similar or deeper than wildtype animals at 10s ISIs but when the ISI is increased to 20s ISI their habituation quickly becomes significantly shallower than wildtype animals. These genes may act in ISI-sensitive habituation processes as their habituation phenotype depends on the ISI. This has implications for how the nervous system integrates stimuli separated by different time intervals.

PS2.24
Vision and photoreception (Part 1)
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Linking eye anatomy to navigation: a biologically accurate model of bees' polarization vision
Many insects rely on patterns of polarized skylight to navigate their habitats. To support this ability, insects evolved specialized ommatidia in the dorsal rim area (DRA) of their compound eyes, which are adapted to detect linearly polarized light across large regions of the sky and form the basis of a polarization compass. The physiological properties of DRA photoreceptors including receptive field sizes have remained largely unstudied. We performed electrophysiological recordings on DRA photoreceptors in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) to measure their receptive field sizes and other visual properties. Our findings provide evidence for photoreceptor coupling, i.e., spatial summation, within the retinal layer of the DRA. We investigate spatial summation as a potential mechanism for increasing the effective receptive field size of DRA ommatidia, a key functional requirement for reliable polarization-based navigation. To link retinal physiology with compass function, we further develop computational simulations of bee polarization vision. We expect photoreceptor coupling in the DRA retina to greatly affect the function of the compass, especially in cloudy sky conditions, where spatial summation can reduce informational noise due to clouds. Our model incorporates species-specific DRA anatomy, including ommatidial optical axis directions, photoreceptor receptive field sizes, and fan-shaped microvillar arrangements. Using our model, we comparatively evaluate polarization compass performance in honey bees and bumblebees, generate testable, species-specific predictions, and identify correspondences between model outputs and existing behavioural data. Our results highlight the importance of a biologically accurate

polarization compass that explicitly incorporates DRA visual properties for understanding insect navigation.

PS1.42

Social behavior and neuromodulation (Part 1)

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Care and cannibalism in *Eleutherodactylus coqui*: tough choices for frog parents

Parents must balance the cost of care with the potential benefits of future reproduction. Filial cannibalism (eating one's own offspring) can allow individuals to recoup resources if the costs of care become too great, or if offspring viability is compromised. Some work has suggested that conserved brain regions and neuropeptides mediate both feeding and parental behavior in a broad range of taxa, and may underlie the switch from caring to cannibalizing. To determine the neural correlates of these disparate behaviors, we studied the common coqui (*Eleutherodactylus coqui*), a frog species that exhibits male parental care and filial cannibalism. To compare neural activity of caring versus cannibalizing parents, we developed a behavioral assay that elicited filial cannibalism in approximately 50% of trials. We used a marker of neural activity (pS6) to compare activation across brain regions in caring, cannibalizing, and control males. We asked how activity of individual brain regions, known to be important in social behavior, is associated with the decision to care or cannibalize. Furthermore, we asked whether activity correlations among brain regions are associated with this dramatic behavioral trade-off. Although there seem to be few differences in neural activity quantified from individual brain regions, correlations of activity between regions may suggest that this behavioral switch is mediated by circuit-level patterns of activation. Our results provide insights into the evolution of neural mechanisms that underlie the trade-offs parents make in balancing current and future reproduction.

PS2.35

Vision and photoreception (Part 1)

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The effect of the whole-genome duplication on vision: opsin expression developmental series and single-nucleus transcriptomics in the common barbel (*Barbus barbus*)

Teleosts stand out among all vertebrates in the number of visual pigments and photoreceptor types. This extraordinary development was also facilitated by the teleost-specific whole-genome duplication that occurred approximately 350 million years ago. Here, we explore a teleost species that has recently undergone whole-genome duplication -

the common barbel (*Barbus barbus*). This event was accompanied by hybridisation (i.e., it was allopolyploidisation) and resulted in 14 opsin genes found in the genome, an unusually high number even among teleosts. We applied comparative transcriptomics across developmental series and generated a single-nucleus RNA atlas of the adult retina to explore how polyploidisation influenced vision in the common barbel. Does the expansion of the opsin gene repertoire result in better visual abilities? We show opsin expression profiles of adult specimens and larvae at different developmental stages. Within three opsin classes (UV-sensitive SWS1, blue-sensitive SWS2, and green-sensitive RH2), we found opsin pairs with a clear temporal division of function - larval and adult genes. Furthermore, the switch in expression from larval to adult gene copy in these opsin classes was synchronised - such an effect has never been reported for a polyploid species. We investigated whether such a division of function can be adaptive. We predict which opsin proteins differ in spectral sensitivity based on the amino acid substitutions in the key tuning sites. Are the absorption spectra of opsins tuned to match the light available in the environment? We localise and visualise opsin gene expression in adult retinae via fluorescence in situ hybridisation, and discuss its functional consequences - namely, whether the presence of more opsins in the retina enhances colour vision or sensitivity of the visual apparatus. Finally, we present the latest results of single-nucleus RNA sequencing performed on the adult retina. We reveal how individual cells utilise vision-related genes acquired by the polyploidisation event. We study the expression of the two simultaneously expressed rhodopsin genes in rods and demonstrate a list of marker genes for rods and cones in this tetraploid species.

PS3.92

Electrosensory systems

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Structure-function relationship of the pacemaker network in weakly electric fish

The Pacemaker Nucleus (Pn) of weakly electric fish *Apteronotus albifrons* is an oscillating neural networkcomprising~150 synchronized neurons. This network controls the firing of an electric organ discharge (EOD) in a 1:1 fashion, producing an oscillating electric field which the fish uses to sense its environment. The EOD fires continuously at frequencies near 1000 Hz with sub-microsecond variation in cycle length, making it one of the most precise biological oscillators known. This extreme temporal precision is transiently modulated on millisecond timescales, to produce communication signals known as "chirps". How this network balances stability and flexibility is still unclear. We hypothesize that the Pn is structured as a "small-world" network, which balances stability and flexibility through clustered and efficient connectivity. We predict that network features will be consistent across individuals, thus providing a framework for understanding network function as well as baseline variability in network structure. To test this hypothesis, we use expansion microscopy (ExM)on whole-mount samples to image the 3-dimensional network structure of the Pn. Expansion of the polymer gel built within samples increases both the optical depth as well as image resolution. From these images, we quantify different network properties, including the degree of clustering and the average distance between neuron pairs. Investigating the structure-function relationship in the Pn may provide insights into the role of network connectivity in optimizing network dynamics.

PS2.65

Spatial orientation and navigation (Part 2)

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A behavioural and electrophysiological framework for studying the neural basis of navigation in freely moving fiddler crabs
Afruca tangeri a semiterrestrial crab that relies on its burrow as the main spatial reference point. At low tide, fiddler crabs emerge from their burrows to feed along the intertidal zone and upon threat detection perform rapid escape responses directed towards the burrow. Field studies suggest that fiddler crabs use an egocentric frame of reference and idiothetic information to update their position and continuously estimate their distance from the burrow during exploration. From an experimental perspective and in contrast to other well-characterised arthropodal model systems, the exoskeletons of crustaceans have the potential to support freely moving in vivo recording approaches to directly monitor neural activity under ethologically relevant behavioural scenarios. Such methodological advances can provide an opportunity to gain mechanistic insight into how internally generated spatial representations of location, heading direction, and distance to goal are formed and guide goal-directed actions.

To implement electrophysiological recordings in freely moving fiddler crabs, we constructed a parametric environment that allows precise control over visual statistics, burrow availability, and burrow location. In this setting fiddler crabs exhibited homing and escape behaviours comparable to those described in the field, allowing precise quantification of their dynamics. We then developed a protocol for chronic extracellular recordings from the central complex using tethered Neuropixel silicon probes, which enabled stable monitoring of neuronal activity during behaviour. Chronically implanted fiddler crabs explored the arena and performed homing and escape responses, and single unit activity was stable across extended recording sessions.

This work provides a foundation for the future use of *Afruca tangeri* a model system to study sensorimotor integration and navigation.

PS1.30
Social behavior and neuromodulation (Part 1)
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Genetic and neural mechanisms underlying evolution of aggressive behaviors
Aggressive behaviors have wide ranging functions, from establishing social dominance to territorial defense. While the aggression is observed across taxa, the mechanisms contributing to natural variation in aggression are largely unknown. The Mexican tetra *Astyanax mexicanus* is a powerful system in which to study the genetic and neural underpinnings of aggression. Two ecotypes of *A. mexicanus* exist, a surface-dwelling form that displays territorial aggression, and multiple populations of a blind cave-dwelling form,

many of which have evolved reductions in aggression-associated behaviors. These two ecotypes are interfertile, and we have performed genetic crosses and mapping to identify the genetic underpinnings of evolved differences in aggressive behaviors between cave and surface fish. Further, we are currently generating an adult brain atlas to investigate the changes in the brain associated with differences in aggression in this species. Together, this work will identify the genetic and neural mechanisms contributing to the evolution of reduced aggressiveness in cavefish, provide candidate genes for targeting via CRISPR-Cas9 to determine their role in aggression, and provide insight into how complex social behaviors evolve.

PS2.52

Auditory systems and acoustic signaling (Part 2)

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A multi-sensor approach for characterizing echolocating bat swarms

Most species of bats heavily rely on their echolocation for sensing their environment, whether hunting, navigation or just plainly avoiding obstacles in total darkness. However, this system of active sensing comes with a drawback widely studied in engineering and signal processing: sensitivity to noise, especially to ìfalseî signals generated by other agents. Due to the high similarity in signal frequencies and acoustic features, the faint echoes of a navigating bat can be overlaid with sounds virtually indistinguishable from the true signal and even be masked entirely by overlaying noise. So, how can bats coexist in large colonies, socialize in flight under complete darkness and even exhibit large swarm movements? So far, most full assessments of this behavior are under laboratory conditions, and field recordings are generally limited to recording tags, which never capture the full swarm behavior.

In my doctoral project, I employ a field-based multi sensor rig to record bat echolocation and flight behavior in groups of free-living bats. I use LiDAR scans of bat swarming sites to get a full 3D representation of the environment and align the scans to the animals in flight trajectories from multiple thermal cameras. Acoustic recordings are made with an extensive microphone rig, mounted on the cave walls and calibrated spatially for accurate capturing of the ultra-sonic sound pressure levels, together with acoustic cameras. Combined, this yields an unprecedented 3D representation of echolocation and movement decisions of every individual echolocator in a swarm. This data offers a unique view into struggles and strategies underlying the seemingly impossible feat of swarm echolocation.

PS2.42

Vision and photoreception (Part 1)

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An active vision behavior in mice that is modulated by environmental complexity in a naturalistic predator-prey task

Using vision to actively search an environment for valuable information is a critical component of survival and everyday function. While these types of behaviors have been observed in the wild, few laboratory studies have elicited and analyzed them. This is due to either key information about rewards or threats being fully visible, or in studies with partial observability, information being static which reduces the need for continual search. We have addressed this by developing a novel behavioral paradigm called cellworld which incorporates reconfigurable obstacles that obstruct vision and a mobile autonomous robot predator that blasts mice with air when close. With cellworld, we found evidence of a novel 'peeking' behavior where mice slow down and look around corners to search for the robot. This behavior was modulated by the structure of the environment. By grouping obstacles together, we report that mice moved further during peeks and did so at locations where there are large changes in the visual field. In contrast, with dispersed obstacles mice moved less during peeks and those peeking locations had no correlation with large changes in vision, suggesting a distinct difference between peeking strategies dependent on environment. We believe these differences may be a function of the structure of visual information in environments, where dispersed obstacles had a more homogenous distribution of changes in vision. To investigate this further, we simulated agents in our task with a state-of-the art reinforcement learning (RL) approach. RL prey agents would perform strategic pauses at transitions of line of sight with the robot that mirrored peeks in real mice. This work therefore establishes a novel animal behavior related to visual information search and highlights a connection between the structure of the environment, decision making, and vision.

PS3.73

Olfaction, taste and chemical sensing

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Parallel antennal lobe subsystems differentially process odor valence in honey bees

A salient feature of animal olfactory systems is the presence of multiple tracts that relay information from early sensory stages to higher-order centers, raising the possibility that parallel pathways process information differentially. In the honey bee (*Apis mellifera*), the two antennal-lobe output projection neuron tracts, L-ALT and M-ALT, offer a system to test whether odor information is differentially routed across pathways. Building on evidence that bees recognize appetitive- and aversive-associated odorants even when presented in mixtures, we ask whether odor information with different hedonic values is segregated across these pathways. To address this question, we combine appetitive and aversive classical conditioning with high-throughput behavioral readouts and automated kinematic tracking, pathway-specific lesions validated by post hoc staining, in vivo calcium imaging, and computational models. Our behavioral data reveal asymmetric odor blocking across reinforcement contexts: a learned appetitive odorant strongly blocks learning about a novel

odorant within a mixture of both, whereas a learned aversive odorant produces a weaker effect, suggesting differential processing in mixtures under different hedonic context. Moreover, severing the lateral tract appears to impair appetitive memory expression, whereas medial tract lesions affect aversive memory expression. Ongoing experiments are testing how inhibitory local interneurons shape the coding of single odorants, mixtures, and pheromones in both subsystems. In parallel, we are developing mechanistic spiking-network models of antennal-lobe circuitry with structured excitation, inhibition, and plasticity to link hypotheses about circuit organization with behavioral and physiological results. Together, these findings support a functional separation between parallel olfactory pathways, with distinct contributions to the processing of odorants with different hedonic values.

PS3.16

Motor systems, sensorimotor integration and behavior (Part 3)

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Open-source tools for modelling the insect sensorimotor loop

Understanding the neural basis of behaviour requires dissecting both sides of the sensorimotor loop: precisely measuring 3D kinematics and accurately modeling the biophysics of sensory perception. In small animals such as insects, this is particularly challenging. Accurately tracking millimetre scale 3D kinematics and simulating complex sensory inputs such as compound vision often require bespoke pipelines (custom code, manually proofreading 2D keypoint detections, integrating disparate tools), diverting substantial effort from scientific questions. To address this, we present two open-source frameworks targeting the critical frontiers of neuroethological data acquisition: sensory simulation and motor quantification.

First, we introduce a robust multi-camera 3D pose estimation pipeline for tracking small, freely moving animals. This platform features automated, real-time spatial calibration with visual feedback and a tracking algorithm that robustly handles noisy, non-proofread 2D keypoints. Beyond integration with tools such as SLEAP and SAM2, it supports a human-in-the-loop mode, enabling precise 3D reconstruction of multiple insects and their physical interactions with the environment.

Second, we present a hardware-agnostic, real-time insect compound vision renderer. Moving beyond standard single-viewpoint ray-casting rendering, this framework utilizes a receptor-centric architecture that supports individual rhabdomere actuation, anisotropic angular sensitivity, and neural superposition. It allows researchers to procedurally generate anatomically faithful eye morphologies and simulate micro-scale active sensing (such as microsaccadic hyperacuity) in real-time, closed-loop environments.

Both tools prioritise developer accessibility and user feedback without sacrificing scientific rigour. By providing these, we aim to equip the neuroethology community with more computational means to decode the sensory drivers and complex kinematics of natural behaviour.

PS2.73

Spatial orientation and navigation (Part 2)

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Visually guided locomotion in the red flour beetle *Tribolium castaneum*

The red flour beetle *Tribolium castaneum* is an insect pest responsible for destroying up to 20% of stored grain in the global south. Groups of beetles collectively destroy grain by moving over and through it in low-light conditions associated with opaque substrates and dark storage areas. Here we explore how light levels and social cues shape locomotion. First, we investigated how adult locomotor behaviour differs between illuminated and dark environments. Beetles were recorded individually (N=15) or in groups of five (N=5) in a circular arena (radius 4.5 cm) for 10 minutes under full illumination, complete darkness (IR only), or a half-covered arena producing a spatial light-dark gradient. Tracks were extracted using automated detection and tracking, and kinematic variables computed from centroid positions. Movement was segmented into bouts (>0.100 cm s?π), enabling quantification of duration, distance, and peak speed. The arena was partitioned into open-field and edge zones to assess open-field running and thigmotaxis. In uniform arenas, illumination altered movement structure but not spatial allocation. Under light, bouts were longer in both open field and edges, and open-field peak speeds were higher, while edge peak speed did not differ. Time spent in open field and edge, as well as fractional occupancy, remained similar between conditions, aside from a modest increase in edge occupancy in darkness. In the split-light condition, beetles showed a clear spatial bias toward the dark region, spending more absolute time there, although proportional occupancy did not reach significance. This bias occurred in both zones, with increased time in the dark-side open field and edge. Movement was reduced on the dark side, with shorter bout durations and lower peak speeds in both regions. The presence of other individuals had minimal impact on locomotion. Overall, illumination modulates the temporal and kinematic structure of locomotion, particularly bout persistence and open-field speed, while spatial occupancy remains largely unchanged in uniform environments. In contrast, heterogeneous environments reveal a strong preference for darker regions, consistent with photophobic behaviour. This work provides a foundation for further study of visually guided behaviours in the red flour beetle.

PS2.17

Motor systems, sensorimotor integration and behavior (Part 2)

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Organization of pretectal optic flow inputs to the zebra finch cerebellum

Optic flow, the global motion of visual features across the retina due to self-motion, is primarily encoded by well-conserved retinorecipient midbrain nuclei in vertebrates, including the pretectal nucleus lentiformis mesencephali (LM). LM sends optic flow information to two regions of the cerebellum- the oculomotor cerebellum (OCb, folia VI-VIII) and the vestibulocerebellum (VbC, foliaIXcd-X). In birds, these pathways are direct projections rather than being relayed by the pons. The pathway to the VbC is implicated in the optokinetic response, in which optic flow causes stereotyped movements of the eyes to stabilize gaze. Midbrain-cerebellum optic flow pathways have also been suggested to be involved in whole body optomotor responses. However, it is unknown if the optic flow

pathways from the midbrain to the OCb, VbC, and their subdivisions carry the same types of optic flow signals and to what extent they are functionally segregated.

I investigated this knowledge gap in zebra finches, which we have been using as a model of visuomotor processing and complex locomotion, using two approaches. First, I performed double retrograde tracing from VbC and OCb to investigate whether LM projections to different cerebellar regions arise from overlapping or segregated populations. This revealed partial segregation of LM neurons projecting to the medial and lateral subdivisions of OCb. Second, I tested adeno-associated virus (AAV) vectors for expressing optogenetic actuators in a pathway-specific manner using Cre-lox expression to independently manipulate projection-target defined sub-populations of LM. From a panel of constructs that differed in viral serotype and promotor, I found most effective expression in LM with AAV5 and humansynapsin1 (hSyn1) promotor. In addition, the combination of AAVDJ/9 and hSyn1 was effective in inducing retrograde Cre expression in LM after injection into the cerebellum. This work supports further investigation and manipulative interrogation of pretecto-cerebellar optic flow pathways and their roles in visuomotor processing.
PS3.39

Social behavior and neuromodulation (Part 2)

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Comparing oxytocin and vasopressin receptor distribution in pregnant and non-pregnant bats

Oxytocin and arginine vasopressin have significant roles in regulating social behaviors. In rodents, oxytocin receptor expression shifts dynamically during the transition to motherhood, and cortical oxytocin signaling enables pup retrieval by enhancing responses to pup calls in the auditory cortex. Despite this growing understanding in rodents, comparatively minimal research has been done on oxytocin receptor (OXTR) and arginine vasopressin receptor (AVPR) distribution in the bat brain. The Seba's short-tailed bat, *Carollia perspicillata*, is a highly social mammal with complex social communication, making it a unique model for investigating the neuroendocrine regulation of maternal behaviors. Here, we examined OXTR and AVPR expression in pregnant and non-pregnant female bats across the auditory cortex (AC) and four brain regions involved in the social behavior network: the amygdala (AMY), prefrontal cortex (PFC), ventral pallidum (VP), and nucleus accumbens (nAC). We found that OXTR and AVPR expression was broadly distributed throughout the five brain regions in pregnant females, whereas expression patterns were more variable in non-pregnant females. These findings provide the first characterization of OXTR and AVPR distribution in pregnant and non-pregnant *C. perspicillata* and establish a necessary foundation for understanding how oxytocin and vasopressin modulate maternal and socially relevant auditory behaviors in bats.

PS2.61

Auditory systems and acoustic signaling (Part 2)

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Characterization of courtship vocalizations in the naked mole-rat
Naked mole-rats, a highly social rodent species, rely heavily on vocal communication across a wide range of social contexts. During courtship, vocal exchanges occur between the queen, the sole breeding female, and breeding males. However, the neural and behavioral mechanisms underlying vocal communication during mate selection remain poorly understood. To investigate the function and structure of vocalization in mate selection and pair bonding, we conducted continuous behavioral monitoring of naked mole-rats as they transitioned to active breeding. We continuously recorded audio from isolated individuals and captured audio and video during pairings. Our study revealed that the onset of courtship behavior coincides with changes in vocalization usage. Both males and females displayed mating preferences, which were reflected in vocalization composition and the number of vocalizations produced. These findings suggest that vocalizations could play a role in mediating partner choice and pair bond formation, providing a foundation for future investigation of the neural circuits governing vocal communication in a eusocial mammal.
PS2.93
Learning, memory and cognition
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Neuromodulatory mechanisms of shelter discovery in mice
Identifying and using shelter locations is critically important for survival and reproductive success of a wide range of species. Many animals are innately driven to find or create burrows and defend them from conspecifics, and behaviours such as courtship, navigation and predator avoidance are strongly modulated by the presence of a shelter. Despite the significant energetic and cognitive resources devoted to securing shelter and anchoring behaviour around it, the neuronal mechanisms that support shelter discovery are not well understood. Here we have investigated the shelter discovery process in mice (*Mus musculus*) and aimed to determine the role of the dorsal raphe (DR) to both shelter learning and the modulation of shelter dependent behaviours.

We first developed a behavioural assay for studying the emergence and modulation of shelter-dependent behaviour using an experimental arena that allows dynamic and automated placement of open and closed shelters while a mouse explores an open environment (AEON)1. To describe behaviour in the presence and absence of shelter and also during shelter discovery, we fitted a Hidden Markov Model to the behavioral data and used it to quantify shelter preference, memory of previous shelter locations, and shelter-dependent exploration in the presence of multiple open or closed shelters.
Using fiber photometry we then measured calcium signals in serotonergic neurons (5HT) of the DR during exploration and shelter discovery. We found that during exploration of novel closed shelters or shelters that mice failed to adopt, the activity of 5HT neurons was transient and similar to activity elicited by generic object exploration. In contrast, during exploration and adoption of novel open shelters we observed large and persistent calcium responses that decayed slowly (approx 20-50 seconds). Additionally, 5HT neurons in the DR responded during visits to previous shelter locations and in response to bedding from their home cage. Current work aims to establish whether 5HT neurons in the DR are required for shelter learning and memory and to establish a mechanistic account of how the serotoninergic system modulates downstream circuits to orchestrate shelter-dependent behaviour.

1 - Campagner et al. 2025, Aeon, doi:10.1101/2025.07.31.664513

PS2.5

Motor systems, sensorimotor integration and behavior (Part 2)

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A semi-intact cephalopod preparation for large-scale neural recording of chromatophore control

Cephalopods are famous for their ability to change skin color rapidly. This behavior is directly controlled by the brain, which sends motor signals to the radial muscle fibers surrounding skin chromatophores, causing them to expand or contract, thereby generating dynamic skin patterns. The skin patterning serves important functions, including camouflage, alarm display, and intraspecific communication. Such pattern generation is largely driven by visual input, but it can also emerge without sensory drive, such as during sleep, suggesting a dream-like state. Understanding how the cephalopod brain generates these patterns requires direct access to neural activity during naturalistic skin displays. However, due to their soft tissues and dense cerebral vasculature, head fixation and brain access are extremely challenging, limiting the application of conventional neural recording methods, such as Neuropixels probes and calcium imaging. Head fixation also largely prevents the expression of normal skin patterns. To overcome these challenges, we developed a semi-intact preparation that preserves the central brain, mantle, pallial nerves, and part of the cerebral circulation. By connecting an artificial circulation system to the carotid artery, we maintained brain oxygenation. Remarkably, this preparation spontaneously displayed skin pattern dynamics resembling those seen in unconstrained animals. In this preparation, we recorded neural activity in the posterior chromatophore lobes of cuttlefish *Sepia pharaonis* during spontaneous pattern dynamics using Neuropixels probes and calcium imaging. Our results begin to reveal how the brain generates mental images without visual input and how these internal representations are transformed into skin patterns across multiple levels of the motor system. This approach provides a tractable platform for understanding how cephalopods produce complex skin patterning behaviors.

PS1.8

Motor systems, sensorimotor integration and behavior (Part 1)

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Optomotor responses of honey bee castes and worker groups are affected by pesticides

Honeybees (*Apis mellifera*) are abundant and effective pollinators contributing enormously to mass production of agricultural crops. They possess visual systems that are highly sensitive to motion, and rely on motion of visual elements, such as wide field motion, to efficiently navigate their complex environment with high precision. Rotational wide field motion stimulates a robust innate response known as the optomotor response, which enables bees to correct for unplanned course perturbations during locomotion. The persistent use of insecticides in seed treatments and bees' exposure negatively impact their ability to engage in these ecologically relevant behaviours. Previous studies showed that neonicotinoids and sulfoxaflor (SFX) impaired walking tracks associated with optomotor responses in honey bee workers. This study investigated responses of different honey bee castes (experiment 1) and worker roles -defined by age (experiment 2), to optomotor stimuli in a 360∞ visual stimulus arena, and how nicotinic acetylcholine receptors (nAChRs) agonists - imidacloprid (IMD) and SFX as well as their mixture (MIX), impair these responses. Bees were tethered on an air-supported ball during presentation of optomotor stimuli consisting of alternating black and white stripes rotating in clockwise and counterclockwise directions. Workers exposed to SFX exhibited significantly faster reaction times and an enhanced preference for a clockwise stimulus direction, while queens in all insecticide-treated groups showed a leftward orientation bias. Drones remained largely unaffected by any of the insecticide treatments. Among the worker role groups, nurses exhibited slower movement and delayed responses compared to foragers and nest bees. We observed no interactive effects between role and insecticide exposure. These findings highlight potential impairments in bees foraging efficiency and orientation, with significant implications for pollination services and pesticide risk assessments.

PS1.28

Social behavior and neuromodulation (Part 1)

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Genome-wide signatures of artificial selection for sociality in zebrafish

Social behavior is a complex trait central to survival, cooperation, and reproduction, yet the genetic mechanisms underlying its evolution remain poorly understood. Artificial selection experiments provide a powerful framework to identify genomic variants associated with behavioral evolution. Here we investigate the genetic basis of sociality using experimentally evolved zebrafish lines selected for divergent social preference. Fish were selected based on social preference assay measuring the mean distance to a social stimuli. Selection produced phenotypically divergent lines, with the social line showing increased attraction to conspecifics as early as in generationF3. To identify genomic variants responding to selection, we combined a genomic and a genetic perspective. At the genomic level, a Wright-Fisher-based framework was used to detect allele-frequency changes exceeding expectations under drift, and phenotype-association tests identified loci correlated with social preference. Integration of these approaches identified 170,621 candidate SNPs across 4,946 protein-coding genes, indicating that adaptation is driven by many small allele-frequency changes distributed across the genome. Local peaks of SNP density suggest focal genomic regions responding strongly to selection. To link these genomic signals to neural function, we integrated the results with brain transcriptomic analyses, including differential gene expression and weighted gene co-expression network analysis (WGCNA). Overlapping candidate genes were enriched for pathways involved in neural development, neuronal signaling, synaptic transmission, and metabolic regulation, suggesting coordinated molecular changes affecting neural circuitry. Together, these results indicate that the rapid evolution of social behavior arises from polygenic genomic shifts coupled with coordinated transcriptomic changes, consistent with large-scale neurobiological rewiring of behavioral circuits.

PS1.59

Social behavior and neuromodulation (Part 1)

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Linking natural alarm calling behavior to auditory activity in wild songbirds

Understanding how brains generate adaptive behavior in natural environments requires studying animals within the ecological and social contexts in which it evolved. Alarm calling is a key mechanism increasing the survival of an animal's social group. However, it remains poorly understood how individual and social factors shape alarm call production and how the brain enables alarm call perception in the wild. Here, we present data from nearly a decade of field research on vocal behavior in a cooperatively breeding, African songbird, the white-browed sparrow weavers (*Plocepasser mahali*).

Using lightweight radio-telemetric microphones fitted to several members of free-living weaver bird groups, we wirelessly recorded the birds' individual vocal activity during natural and simulated predation events. Alarm calls emitted during these predation events were analyzed in relation to each bird's sex and social status, and in relation to environmental factors, such as time of day, and group size. Preliminary results indicate that alarm-calling behavior varies across individuals and contexts, suggesting that both social and environmental factors may shape vocal responses to predation risk.

In a subset of birds, we surgically implanted electrodes in two auditory brain areas, the 'cortical' Field L and the thalamic Nucleus Ovoidalis, to telemetrically record extracellular neural activity in parallel to the conspecifics' alarm calls during natural alarm calling events. Despite frequent alarm calling of nearby conspecifics, many implanted individuals showed limited auditory responses, suggesting context-dependent or selective listening strategies in the wild.

These results highlight how social structure and ecological demands shape both communication behavior and auditory processing. This work underscores the necessity of integrating natural behavior to test laboratory-derived hypotheses and adds to a growing body of research on neural mechanisms underlying natural behavior.

PS2.72

Spatial orientation and navigation (Part 2)

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Temporal difference learning for navigation in an insect mushroom body circuit

Bees are among the master navigators in the insect world. In the realm of robotic navigation, Reinforcement learning (RL) algorithms have proven equally powerful. Here we present a neural implementation of temporal difference (TD) RL for navigation, based on the architecture of the learning and navigation centers of the insect brain: the mushroom body

(MB) and the central complex (CX), respectively, which are largely conserved across species. We propose that the same basic architecture can support increasing navigational complexity by scaling up the number of MB output neurons (MBONs) and thus the complexity of the available action space: in *Drosophila* with only ~40 MBONs per hemisphere, a small subset of MBONs would be sufficient to encode a coarse heading preference; in honey bees, the vastly larger MBON population (~400 per hemisphere) might support a richer action space, encoding distinct vector goals and providing a potential link between landmark and vector-based navigation strategies. In the MB, dopaminergic neurons (DANs) - mediating absolute reinforcements or prediction errors -gate plasticity at KC?MBON synapses, which transform sensory inputs into behaviorally relevant outputs. This makes the MB a natural candidate for TD-RL, where temporal bootstrapping of reward predictions is used to estimate a value function over (sensory) states. However, there are two key differences between current MB models and TD-RL. First, DAN activity is treated as a reward prediction error at best, lacking the bootstrapped future value term required for true TD learning. Second, MBON output is usually identified with learned valence, leaving unresolved how this is converted into a specific navigational action. We present an extension to current MB models where additional recurrent MBON?DAN feedback implements the bootstrapped TD error, and MBONs encode both valences (‘values’) and navigation-relevant actions via their projections into the CX. The KC population provides a shared state representation, while action-specific KC?MBON weight vectors implement a learnable ‘policy’. This reframes the MB-CX circuit as a biologically grounded TD architecture, bridging insect neuroethology and modern reinforcement learning.

PS1.47

Social behavior and neuromodulation (Part 1)

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Mapping the clonal raider ant brain at synaptic resolution

Ants have been studied extensively due to their advanced social structure and communication strategies, though the neurobiological basis of these behaviors is not well-understood. The clonal raider ant *Ooceraea biroi* is particularly amenable to investigating the neural circuits underlying social behaviors, as it is uniquely experimentally tractable and genetically accessible. To characterize the structural connectivity of the ant brain, we imaged a whole brain at synaptic-level resolution using transmission electron microscopy (TEM). This whole-brain EM volume allows us to reconstruct neurons, identify synapses, and ultimately create a complete wiring diagram, or connectome, of the ant brain. To accelerate circuit reconstruction, we applied machine learning based approaches to segment neurons and predict synapses, followed by ongoing manual proofreading to ensure accuracy. To facilitate integration with existing resources, we registered the EM volume to a reference light-microscopy brain atlas of the clonal raider ant, enabling correspondence between cells identified across imaging modalities. Given the central role of olfaction and pheromone-mediated communication in ant social behavior, and the dramatic expansion of odorant receptor genes and olfactory glomeruli in ants, our initial reconstruction efforts focus on elucidating the wiring logic of the olfactory system. More broadly, this segmented whole-brain EM dataset, and the eventual complete connectome, provide a foundational resource for studying the neural circuits underlying behavior in the clonal raider ant. This resource will support ongoing efforts to learn how neural circuit organization supports collective behavior, division of labor, and pheromone-guided decision-making in social insects.

PS1.14

Motor systems, sensorimotor integration and behavior (Part 1)

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A modular building-block model of marmoset vocalizations

Primate vocal production is increasingly recognized as a dynamic and flexible process, challenging traditional views that emphasize stereotypy and fixed signal forms. In common marmosets (*Callithrix jacchus*), our previous work identified distinct acoustic subunits within long-distance phee calls, indicating that these calls are composed of smaller building blocks rather than existing as holistic fixed action patterns. This finding suggests a more modular organization of vocal production than traditionally assumed. In the current study, we investigate whether this subunit-based compositional structure extends across call types. We analyzed acoustic data from freely moving marmosets with recordings under experimentally controlled auditory perturbations. Our results reveal consistent patterns and temporal dynamics that align with the presence of discrete vocal units, supporting the hypothesis of a modular vocal architecture. These findings have important implications for understanding the neural substrates that enable such structured vocal production and raise intriguing questions about the sensorimotor mechanisms underlying vocal flexibility in primates.

PS3.94

Electrosensory systems

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Electric field detection and electrotaxis in the *Drosophila* larva

Electrosensation is a critical sensory modality for social communication and foraging across diverse animal species, yet its presence in *Drosophila* has remained largely unexplored. Using a novel assay incorporating finite-element modeling of electric potentials, we demonstrated that *Drosophila* larvae exhibit robust electrotaxis, migrating toward the cathode (negative potential) in uniform electric fields (?0.4 V/cm) and rapidly adjusting to changes in field orientation. Larvae use active sampling through stops and lateral head casts to reorient. Following a behavioral screen, we identified a small subset of gustatory neurons in the larval head as necessary for electrotaxis. Calcium imaging revealed that a Gr66a-positive neuron is strongly activated when the cathode is positioned posterior to the head and shows no response when the cathode is anterior, suggesting larvae move toward the cathode to minimize aversive sensory input. These results provide new evidence for electrosensation in invertebrates and establish *Drosophila* as a tractable model system for investigating the molecular and neural mechanisms underlying this sensory modality.

PS2.43

Vision and photoreception (Part 1)

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Behavioral consequences of genetical lesions of the tectofugal pathway in chicken

In vertebrates, retinal information is conveyed via two major projection pathways to the forebrain: the tectofugal and the thalamofugal pathway. While the projection strength of either pathway varies across vertebrate species, both play important roles in guiding visual behavior, with partly conflicting evidence from behavioral, physiological, and lesion studies. Here, we took advantage of a genetically manipulated chicken with the deletion of a transcription factor (AP-2?) that has a highly restricted developmental expression in the retina and midbrain sensory nuclei. Interestingly, the projection from the optic tectum to the next nucleus of the tectofugal pathway is almost completely abolished during development, leading to a disruption of the tectofugal information transfer to the forebrain. We studied the behavior of knock-out chicken with deep-learning-based pose estimation, as well as startle responses and auditory brainstem responses. AP-2? KO chicks had difficulties in pecking food and had to be fed for the first several days, until they had learned to peck grains. The animals showed reduced social proximity and decreased locomotor activity, including fewer high-speed movement bursts, while maintaining normal occupancy of food and water areas. KOs exhibited faster reactions to visual startle stimuli, but a markedly reduced probability of acoustic startle despite normal response latencies when a startle occurred. ABR thresholds to tone bursts (0.5-3 kHz) were unchanged, indicating preserved peripheral hearing; however, late ABR components consistent with midbrain auditory generators were strongly attenuated. OCR tracking was selectively impaired at slow visual motion. Together, these data indicate that AP-2? is critical for proper development of midbrain sensory processing-particularly auditory midbrain function-while leaving peripheral hearing intact and producing selective deficits in low-speed visuomotor stabilization. This KO model will allow us to investigate behavior and forebrain processing in the absence of the ascending tectofugal projection.

PS2.57

Auditory systems and acoustic signaling (Part 2)

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Old-world roundleaf bats as a model for studying vocal frequency control of mammals

Much of the information in human speech is encoded by modulating the energy distribution of spectral content. Similarly, a growing number of animals demonstrate flexible control over the spectral parameters of their vocalizations. Some echolocating bats, in particular, achieve extremely rapid and highly precise frequency control-a capability unparalleled by most other species. In this presentation, I introduce Old World leaf-nosed (roundleaf) bats (Hipposideridae) as a model for understanding the neurobiological mechanisms of precise frequency control in mammals. We first demonstrate that Pratt's roundleaf bats dynamically

adjust their frequency precision while pursuing insect prey, achieving a best average precision of 0.15% of the echo frequency. This is approximately 5-10 times more precise than the pitch control of professionally trained human singers. Using a high-speed FPGA system to provide frequency-shifted playback to vocalizing bats, we found that these animals rely on sensory prediction errors to fine-tune call frequency, a process computationally equivalent to the mechanisms governing human speech. Finally, we established a chronic electrophysiological setup to record extracellular single-unit activity in the auditory midbrain (inferior colliculus) of freely vocalizing bats. We show that many midbrain neurons encode the peak frequency of both self-emitted calls and acoustic playbacks. Interestingly, a specific population of neurons exhibited distinct frequency tuning profiles when responding to self-emitted calls versus calls from conspecifics or pure tones. Together, these data suggest that roundleaf bats facilitate integrated research from behavior to neuronal activity, representing a promising model for understanding vocal production control in mammals.

PS1.23

Motor systems, sensorimotor integration and behavior (Part 1)

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Reciprocally connected ascending and descending neurons contribute to flight performance.

Corollary discharge circuits send predictive motor signals to sensory and other motor networks to modulate processing, thereby adaptively coordinating sensorimotor function. However, the neural sources of their motor-related information and those sources' role in behavior are often uncharacterized. The mesothoracic ascending histaminergic neurons (MsAHNs), are a pair of corollary discharge neurons that activate prior to and during wing movement and project their predictive motor signals to gustatory, auditory and visual processing neuropil in the brain. To characterize the sources of motor information that the MsAHNs integrate, we used connectomics, immunolabeling, and behavioral assays in *Drosophila melanogaster*. A full CNS volume reconstruction indicates that a descending neuron cell pair, DNg32s, form a reciprocal synaptic relationship with MsAHNs. DNg32s provide ~226 synaptic outputs to the MsAHNs in the VNC and in turn receive ~40 synaptic inputs from them in the brain. Immunolabeling confirmed that the DNg32 are cholinergic. To characterize their role in flight performance, we disrupted both MsAHN and DNg32 function by expressing UAS -Botox or -Reaper in separate experiments in restricted Gal4 lines. Flies were tested on odor-guided flight to a chemoattractant as well as voluntary flight takeoff performance. Both UAS constructs increased the flies' ability to reach their target for both MsAHNs and DNg32s. Flies were also challenged to make voluntary flights which were recorded using high speed imaging. Video analysis of UAS-Botox and -Reaper crosses showed a significantly increased number of spontaneous flights in both lines but only reaper significantly disrupted flight performance in MsAHNs. Both UAS constructs

disrupted flight performance in DNg32. Finally, we crossed UAS-Chrimson in DNg32 flies and observed a significant reduction in likelihood of voluntary flight in the presence of red light. Overall these results support the hypothesis that both MsAHNs and DNg32s play a role in flight performance. Future physiological studies will characterize their reciprocal interaction.

PS3.80

Olfaction, taste and chemical sensing

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Influence of life history on alarm responses in larval Dendrobatidae

Alarm pheromones are secreted from injured animals and used to alert conspecifics of impending danger. While there has been substantial work to characterize how the brain detects and responds to alarm pheromones in species that interpret them as honest danger signals, the role that life history has in the interpretation of the signal is unclear. Tadpoles in the Dendrobatidae clade offer a unique lens to address this question because species exhibit either gregarious (*Allobates femoralis*), cannibalistic (*Ranitomeya imitator*), or plastic (*Phyllobates terribilis*) behaviors. With this behavioral difference, alarm pheromones derived from injured conspecifics could represent food resources or danger based on the receiver. We tested the hypothesis that life history tunes the responses of tadpoles to alarm cues and predicted that cannibalistic tadpoles would hunt while gregarious species would avoid the cue. We found that gregarious A. femoralis tadpoles decrease their movement and freeze. Coupled to this behavior, we see enrichment of SLC6A1, a gene that encodes a GABA transporter, in cells that were activated in response to alarm pheromones. In *P. terribilis*, animals increase their movement and spend more time near the alarm pheromone. Larval *P. terribilis* show depletion of genes that encode neuropeptide Y receptors and genes associated with acetylcholine signaling. This work demonstrates how life history affects an animal's decision to either approach or avoid alarm pheromones and the potential role that inhibitory and feeding related neural circuits play in this decision will continue to be investigated.

PS2.33

Vision and photoreception (Part 1)

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Comparative analysis of polarization vision in mosquitoes

Many insect species possess specialized photoreceptors and neural circuits capable of detecting the orientation of the electric field vector of light, i.e., light polarization. In contrast, humans are essentially blind to this modality of light. In insects, polarization cues enhance contrast vision, support object detection, and aid environmental assessment, contributing to behaviors such as navigation, habitat detection, host recognition, foraging, and collision avoidance. Despite its ecological relevance, the role of polarization vision in mosquitoes remains largely unexplored. Evidence for polarization sensitivity in mosquitoes mainly comes from optomotor assays in *Aedes aegypti*, which show significant responses to moving polarization patterns. However, whether similar capabilities exist in other medically relevant mosquito taxa is unknown. We developed a comparable optomotor drum setup to investigate polarization vision across multiple species. As a baseline, we quantified the visual acuity of *Anopheles stephensi*, an important malaria vector, by measuring optomotor responses to rotating black-and-white stripe patterns of different widths. Consistent with expectations based on the larger interommatidial angle of *Anopheline* species compared to *Aedes*, *An. stephensi* displayed lower visual acuity. While 100% of *Ae. aegypti* males and 80% of females respond to a stripe width of 9∞, none of the tested *An. stephensi* males (n=12) or females (n=12) responded to this pattern. The same individuals showed robust optomotor responses to a wider 22.5∞ stripe pattern (8 RPM; response rate 100%). Building on these findings, we have developed matching polarization patterns, enabling us to establish polarization vision in *Anopheline* species. This system provides controlled, reproducible, and uniform visual stimuli, enabling comparison among mosquito taxa. Our approach establishes a framework to advance the understanding of polarization processing in mosquito visual ecology.

PS2.34

Vision and photoreception (Part 1)

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A distinct state of arousal affects visual acuity in the paper wasp *Polistes fuscatus*

Fast responses to threats require coordinated changes across posture, perception, and physiology. These effects of the so-called fight-or-flight response are well characterized in vertebrates. Insects show clear postural and physiological responses to threats, but the effects on sensory processing are poorly understood. Paper wasps have to defend their open nests against predators and conspecifics and previous studies have demonstrated a distinct fight-or-flight like state of aggression expressed through wing flaring in this species. We chose the paper wasp *P. fuscatus* as our model system to test how an internal state affects visual processing. To do so we built an immersive 3D virtual environment to control the visual stimuli surrounding a wasp nest. Foundresses reliably responded to moving stimuli presented on the screen. Responses were more likely when foundresses were in aggressive postures, indicative of increased motivation to defend the nest. We reliably induced aggressive posturing by vibrating the nest. Wasps in aggressive states responded to smaller moving objects, demonstrating increased visual acuity (1.4∞ instead of 2.1∞ visual angle). This shows that early visual motion processing changes in a state dependent way. This state switch allows single foundresses to economically defend their nests by remaining in a low arousal state most of the time and entering into a state of higher vigilance and arousal when necessary. We comprehensively show that visual processing changes during a fight-or-flight state in paper wasps highlighting an important evolutionary convergence to vertebrate sympathetic nervous system function.

PS2.8

Motor systems, sensorimotor integration and behavior (Part 2)

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Evidence of a shared acoustic-respiratory relationship across birds from a deep learning framework

Birdsong has many similarities to human speech in how it is learned and produced. For this reason, birdsong serves as a valuable model for understanding the generation of complex motor behaviours. In birds, sound is produced by passing air through the trachea, which vibrates the syringeal muscles in the syrinx. These observations indicate that at least two parameters control sound production: air pressure and syringeal muscle tension. Previous theoretical studies have shown that diverse syllable types can be produced by varying syringeal tension and respiratory pressure in a model. In addition, in different zebra finches, acoustically similar syllables have similar underlying respiratory patterns. Despite previous studies suggesting a consistent relationship between acoustic properties and respiratory pressure across individuals, this question has limited direct experimental evidence. Here, we addressed this question by recording song and respiratory pressure in Bengalese finches, a songbird species with complex song sequences. Syllables from each bird were segmented, and 9ms segments of these syllables and the associated mean respiratory pressure for that segment were used to train different deep learning models. The models were then used to predict respiratory pressure for novel syllables from the same bird and from novel birds not used in the training phase. Models trained on one bird predicted respiratory pressure for untrained syllables from the same bird with above-chance accuracy. They also predicted respiratory pressure patterns from other novel birds, also with above-chance accuracy, but these were lower than those for data from the same bird. Predictions on syllables from novel birds improved when we trained models with data from multiple birds, but accuracies were still lower than those for the same bird. Overall, the results indicate a largely shared acoustic-respiratory relationship across different birds, with some notable individual differences.

PS2.28

Vision and photoreception (Part 1)

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A moth's point of view – form and function guide innate flower symmetry preference

Floral visual cues are key to aiding flower recognition for pollinators and may have developed closely with pollinator visual systems. In insects, innate preferences for characteristic floral colour or pattern cues can guide naïve foragers to their targets. Symmetry, a remarkably conserved floral feature, is suggested to be one such cue since pollinators such as bumblebees and honey bees can detect symmetry and associate it with food rewards. But whether insects innately use symmetry to guide foraging choices, and if these preferences are also linked to foraging benefits, remains uncertain. We investigated naïve symmetry choice and its foraging function in the hummingbird hawkmoth (*Macroglossum stellatarum*). In addition to using visual cues for flower choices, these moths

feed while hovering, making symmetry a potentially valuable visual cue for flight and proboscis control while foraging. In choice assays using typical floral symmetries: bilateral and radial, moths preferred bilateral over radial symmetry when they were displayed in the horizontal plane. However, when the same patterns were displayed vertically, choices reflected preferences for specific feature positions within a pattern, rather than overall symmetry. Using pose estimation, we tracked freely foraging moths to test whether the preferred patterns benefited motor control. The preferred horizontal patterns directed efficient proboscis contacts with the nectary, while the preferred vertical patterns guided stereotyped approach flights, increasing the chance of nectary contact. When analysing flowers visited by hawkmoths in nature, we did not recover a symmetry-display association; we did, however, identify a large group of flowers with feature positioning matching the naïve preference of moths in the vertical display. Together, our findings demonstrate display-dependent visual pattern selection and highlight how visuomotor strategies in nectivorous insects may have strongly adapted to flower features.

PS2.100

Metabolism, biological rhythms and homeostasis

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Circadian microbial oscillations in *Carollia perspicillata*

Circadian oscillations in the gut microbiome contribute to host metabolic and immune regulation and are well characterized in laboratory rodents and humans. In these systems, microbial rhythmicity is closely linked to feeding cycles and host circadian gene expression, yet most evidence derives from mammals with slow gut transit and fermentation-rich digestive physiology. Whether comparable temporal organization occurs in mammals with rapid transit and reduced fermentation capacity remains unclear. Here, we characterized diel gut microbiome changes in Seba's short-tailed fruit bat (*Carollia perspicillata*), a species with streamlined digestive physiology associated with powered flight. Across two independent cohorts sampled repeatedly during inactive and active phases, we quantified microbial composition and functional potential alongside automated behavioral tracking. We identify pronounced phase-dependent restructuring of the microbiome, with distinct taxa enriched during resting and active periods. Several taxa commonly associated with pathogenicity in other mammals exhibit stable rhythmic abundance in bats, indicating that temporal organization is maintained within this rapid-transit, Proteobacteria-rich system. These findings extend circadian microbiome research to a physiologically distinct mammal and provide a foundation for future mechanistic investigation of host-microbe temporal dynamics.

PS2.26

Vision and photoreception (Part 1)

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Context-dependent loom responses in snapping shrimp

Many animals rely on their visual systems to gather, filter, and process information from the environment that is critical for survival. Some animals have visual systems adapted for rapid temporal sampling, which allows them to resolve fine details in visually-complex environments. The bigclaw snapping shrimp, *Alpheus heterochaelis*, is a marine crustacean found in oyster reefs that has the fastest recorded temporal sampling rate of any aquatic animal. Such fast vision may help *A. heterochaelis* navigate their visually-complex habitat while avoiding predators. We investigated visually-guided behaviors in *A. heterochaelis* using a behavioral loom assay where expanding discs represent an approaching predator. We found *A. heterochaelis* primarily respond by freezing at smaller subtended angles with slower loom speeds, which plateaued after a speed of 7 cm/sec. These results suggest a context-dependent loom response, where loom speed changes the visual threshold for a behavioral response. Next, we asked how loom stimuli at varying flicker rates influence loom responses. This question is crucial to understand if fast temporal sampling rates influence the behavioral output in *A. heterochaelis*. We found that *A. heterochaelis* demonstrated longer freeze durations when looms played at 240 Hz compared to 120 Hz. These flicker rates straddle the known temporal sampling abilities of A. heterochaelis and may indicate a behavioral shift with stimuli of varying rates. This investigation marks one of the first attempts to find the relationship between visually-guided behaviors and temporal resolution in an animal with extremely fast sampling rates. Our findings illustrate how time-dependent aspects of vision are shaped by ecological pressures, providing insight into sensory adaptation in marine invertebrates.

PS3.101

Methods

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Whole-brain gene expression and neuronal activity at cellular resolution in behaving zebrafish

The brain's remarkable capabilities rely on both the molecular properties of individual cells and their interactions across brain-wide networks. However, relating gene expression to activity in individual neurons across the entire brain has remains elusive. Here we developed an experimental-computational platform: Whole-brain neuronal Activity and RNA Profiling (WARP), that integrates calcium imaging, expansion-assisted spatial transcriptomics, and the registration of these two modalities at the cellular level. Through joint analysis of brain-wide neuronal activity during multiple visually evoked behaviors, cellular gene expression, and anatomical localization, we identified functions of over 2,000 molecularly defined subpopulations, including luminance coding in a cckb-pou4f2 midbrain population and motor integration in neurons co-expressing the genes glyt2-gfra1a. WARP also revealed that most gene-matched neurons showed stronger local activity correlation, implicating gene expression in functional organization. By bridging for the first time molecular identity with neuronal dynamics at the whole-brain scale, WARP establishes a foundational platform and open-access dataset for cross-experiment discovery, high-throughput function-to-gene mapping, unification of cell biology and systems neuroscience, and scalable circuit modeling at the whole-brain scale.

PS3.93

Electrosensory systems

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Dynamic social interactions constrain spatial coding in the electrosensory system

Sensory-guided behavior relies on signals that vary dynamically in space and time. Characterizing this spatiotemporal structure is crucial to understanding the tasks sensory systems face. In weakly electric fish, social interactions rely on electrosensory signals to guide movements relative to other individuals. A ghost knifefish's continuously generated electric field allows conspecifics to detect and locate them. Casual observations of their behavior, chasing and interacting with one another, hint at a highly sensitive system that extracts accurate spatial information even when signals are weak or targets move rapidly. While the remarkable sensitivity of this system has been explored using prey-like stimuli, rapidly moving conspecifics add an additional layer of complexity. Here, we examine how the spatiotemporal dynamics of social interactions affect the accuracy of spatial coding in the early sensory system. We hypothesize that feedforward rate-based coding does not provide sufficient spatial information under the dynamic conditions of natural social interactions. We first record dyadic interactions to characterize the range of spatial dynamics. These interactions define the structure of natural sensory inputs, which we reconstruct using a model of ghost knifefish electric fields. These inputs are then provided to a full-scale population model of electroreceptors and primary sensory neurons (ELL pyramidal cells). Based on these responses, we evaluate how spatial information supports the observed behaviors. We show how these sensory responses support spatial localization under natural conditions, and identify where additional mechanisms are likely required to account for the observed behavioral precision.

PS2.99

Metabolism, biological rhythms and homeostasis

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Social physiology: human homeostasis is more efficient in social proximity

Homeostasis is a fundamental biological principle conserved across species, typically conceptualized as an individual-level regulatory process under natural selection. However, this individual-centric framework has obscured the possibility that, in social animals, sociality may itself be conserved in part because it promotes homeostatic efficiency. While the evolutionary role of sociality through cooperation or threat buffering is well recognized, it has not been tested whether social proximity enhances core homeostatic regulation.

Here, we examined whether social proximity improves physiological regulation in humans across multiple homeostatic domains. In preregistered experiments, human adults completed metabolic, thermoregulatory, and stress challenges both alone and in the presence of a close partner. Following a carbohydrate challenge, glucose homeostasis was more efficient in the Social compared to the Alone condition (n = 108), characterized by lower perturbation amplitude and faster recovery. This effect replicated in thermoregulation during a cold exposure (n = 116), and in sympathetic arousal during stress in adults (n = 115) and infants (n = 56). Across systems, social proximity consistently reduced physiological perturbation and accelerated return to baseline, independent of stress reduction or physical touch. These findings demonstrate Social Physiology: a cross-domain principle in which physiological homeostatic is enhanced by social proximity. Beyond behavioral and ecological advantages, Social Physiology reveals a robust psycho-biological benefit of sociality that may contribute to the evolution and maintenance of social bonds.

PS3.47

Vision and photoreception (Part 2)

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Dim lights, big insights: luminance adaptation in nocturnal moths

Nocturnal animals must collect sufficient light to subserve visually guided tasks. This is complicated by luminance conditions which can change rapidly by several orders of magnitude due to changing cloud cover and moving between differently shaded areas, with artificial light sources further compounding the challenge. A nocturnal moth, *Deilephila elpenor*, uses both neuronal and optical mechanisms to control how much visual information it integrates to overcome these conditions, showing increased spatial and temporal integration of visual information in response to decreasing luminance. How this adaptation to luminance evolves over time is as yet unexamined.

We designed a visual stimulus system using a pair of moving, variable-density neutral density filters, which coordinate to evenly and dynamically attenuate the luminance of a projector's output. Responses to these visual stimuli were recorded extracellularly from lobular neurons responsive to optic flow, using a multi-electrode probe. Before and after changes in luminance, we vary the spatial and temporal features of visual stimuli, characterising how the integration of those modalities adapts over time. Preliminary data suggests that neuronal adaptation to decreases in luminance is many times slower than adaptation to increases in luminance (10-15 minutes vs 2-3 minutes). Further stimuli test whether the known spatial and temporal integration changes occur on these same timescales or independently.

The asymmetry of dynamics of luminance adaptation may subserve greater flexibility to the sudden increases in luminance when finding better-lit locations with less shade, while maintaining the slower changes resulting from overall environmental changes. Characterising the dynamics and processes contributing to luminance adaptation will reveal the mechanisms and efficacy of nocturnal insects' adjustments to rapid luminance changes, both in natural and in artificially lit surroundings.

PS3.67

Olfaction, taste and chemical sensing

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Heat stress during pupal development affects olfaction in the armyworm moth

Sedentary insect life stages may be more susceptible to stochastic weather events such as heat waves. Past studies have shown that armyworm moths that have been heat stressed for 48 hours as pupae had lower mating rates, mediated primarily by sex pheromones. I conducted electrophysiological and molecular experiments to test whether the same 48-hour heat stress conditions affected chemical sensing in antennae. I also tested behaviours in males associated with mate finding. I found that male antennal responses to female pheromone components were reduced by pupal heat stress. However, analysis showed that these differences were not driven by changes in receptor expression. In the wind tunnel, treated males showed no significant behavioural differences from control males, in response to pheromone sources. These findings highlight a possible disconnect between microscale physiology and real-world behaviour. I will discuss implications of these findings through both neurological and ecological lenses.

PS2.85

Learning, memory and cognition

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Associative learning enhances odor generalization in the locust antennal lobe

Associative learning allows organisms to adapt behavior by generalizing information from reinforced experiences. While many species require repeated exposures to form associations, locusts rapidly learn and generalize from only a few interactions. This capability arises from the complexity of the locust olfactory system, particularly the Antennal Lobe (AL), where a plastic network of projection and local neurons processes odor information. Studying this system provides insight into biological mechanisms of associative learning that may inform machine learning models capable of rapid generalization. Here, we examined how AL neuronal responses to a target odor change after reward-based associative learning and how this learned association generalizes to other untrained odors. Odor-evoked activity was recorded from AL neurons before and after training. Five odors were presented to the locust: a target odor, a chemically similar odor, a perceptually similar odor, an odor control, and a solvent. Baseline responses were measured prior to training, after which the locust was trained to associate the target odor with reward. Odors were then re-presented while maintaining the AL electrode position. Population analysis revealed that associative learning significantly increased neuronal spiking to the trained odor as well as to the chemically and perceptually similar odors, while controls remained largely unchanged. Among neurons showing strong increases in spiking to the trained odor, there was a 66% probability of increased responses to the chemically similar odor and a 50% probability for the perceptually similar odor. These activity patterns occurred throughout odor presentation, revealing differences in learned generalization between transient and steady-state stimulus periods. Together, these findings demonstrate the locust's capacity for rapid associative learning and generalization across odors, providing insight into how neural responsiveness evolves during stimulus processing.

PS3.70

Olfaction, taste and chemical sensing

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Detection of multiple PFAS in environmental samples using a neuron-based gas sensor

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants widely detected across ecosystems, leading to extensive human exposure. Their chemical stability and accumulation at trace levels present major challenges for environmental monitoring in complex natural samples that contain numerous background chemicals. Although analytical techniques such as liquid and gas chromatography-mass spectrometry provide high chemical specificity, these approaches lack the broad sensitivity and real-time sensing required for diverse PFAS identification at environmentally relevant concentrations (i.e., parts-per-trillion). These limitations motivate the development of alternative sensing strategies capable of identifying PFAS in chemically complex environments. Biological olfactory systems provide a powerful model for chemical sensing, as they are naturally optimized to identify target compounds among variable background odors. In insects, particularly locust, chemical information is encoded by populations of olfactory neurons and transformed by neural circuits into stimulus-specific spatiotemporal patterns, enabling robust discrimination of chemicals at trace concentrations. Incorporating biological olfactory neural processing offers a complementary sensing strategy to existing technology. Here, we apply a neuron-based bioelectronic sensor to evaluate PFAS detection in environmental matrices. Extracellular neural activity recorded from locust antennal lobes was used to compare environmental water samples collected from a Michigan wetland with and without additions of PFOS and PFOA at environmentally relevant concentrations (i.e., ppt). Despite the presence of naturally occurring background chemicals, population-level neural responses enabled reliable discrimination of PFAS-contaminated samples using a linear classifier. These results highlight the robustness of biological neural sensing for real-time PFAS detection under realistic environmental conditions.

PS3.56

Vision and photoreception (Part 2)

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A neural circuit for stereopsis in the fly

Animals can combine information from both eyes to generate a rich three-dimensional percept of the world (stereopsis). Neurons tuned to binocular depth cues have been identified in many species (including insects), but uncovering the underlying circuitry responsible for this computation remains a challenge. Theoretical models suggest that binocular distance tuning arises via feed-forward circuits; however, testing this hypothesis requires a complete map of the connections that combine information from both eyes. Here, we analyze and interpret two whole-brain wiring diagrams (‘connectomes’) of the fly (*Drosophila melanogaster*) to uncover a complete circuit that supports distance-dependent behaviors driven by stereopsis. We focus on LC14 neurons, which have a unique

architecture connecting the lobulae of both optic lobes, and their upstream and downstream circuitry. In contrast to classical feed-forward models, we find that stereopsis in the fly arises via a recurrent circuit between the optic lobes that convolves the images from the two eyes. Modeling shows that this recurrent architecture provides more robust distance and size tuning than a purely feed-forward circuit. We map the 3D receptive fields of LC14 neurons to show that stereovision in the fly is most sensitive to millimeter-sized objects at close range (<5 mm), suggesting that binocular distance estimation might support near-range behaviors directed towards other flies. Corroborating this, we find that a subset of LC10a neurons (tuned to visual features of conspecifics) in the binocular zone receive input from LC14, and that these binocular LC10a have distinct targets in the central brain that drive specific sensorimotor transformations for near-range social behaviors. Genetic silencing of LC14 impedes male courtship, and two-photon calcium imaging in behaving males reveals they are tuned to fly-sized objects in the binocular visual field. Together, we have used the fly connectome to discover a synaptic-resolution circuit mechanism for computing distance from binocular cues and uncovered a putative role for binocular vision in patterning social behavior.

PS2.46

Auditory systems and acoustic signaling (Part 2)

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Preserved auditory function with aging in Japanese house bat: ABR and hair cell analysis

Bats are known for their longevity despite their small body size and rely on high-frequency sounds for navigation and foraging. Hearing is generally known to be susceptible to aging. Therefore, bats, which depend on hearing throughout life, may possess unique resistance mechanisms. In this study, we compared auditory function between young and old awake Japanese house bats (*Pipistrellus abramus*) by measuring auditory brainstem response (ABR) using 11 frequencies from 10 to 80 kHz. ABR were obtained by averaging evoked potentials in inferior colliculus. We evaluated latencies and hearing thresholds calculated from the ABR between age groups. As a result, no significant differences in thresholds were observed between the young and old groups, suggesting that bats may maintain auditory function despite aging, even though they are routinely exposed to intense echoes and vocalizations from conspecifics. To clarify the mechanism underlying the maintenance of auditory function, we additionally performed staining of cochlear hair cells and compared the results with C57BL/6 mice that exhibit age-related hearing loss. In the cochlea, different regions respond to different sound frequencies. Therefore, the cochleae of bats and mice were divided into three regions (apex, middle, and base) and stained separately. The numbers of hair cells in each region were analyzed. As a result, bats exhibited the highest number of outer hair cells in the base, which was slightly higher than that observed in mice. Also, no interspecific differences were detected in the other regions. Overall, our results suggest that Japanese house bats preserve auditory function with age. Also, its high auditory capability may be supported by an increased number of hair cells in base, where hair cell loss is often observed in age-related hearing loss, as well as by molecular-level mechanisms such as differences in cell morphology and the expression of specific proteins.

PS3.68

Olfaction, taste and chemical sensing

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Snapping shrimp use distance and contact chemoreception to identify mutualistic gobies

Animals in mutualistic partnerships rely on their sensory systems to identify partners after separation or risk losing cooperative benefits. The snapping shrimp *Alpheus bellulus* and the goby *Cryptocentrus cinctus* engage in mutualistic partnerships where *A. bellulus* provides both partners with shelter and *C. cinctus* warns of predators. Since both partners leave the burrow to forage, it is imperative that they identify each other upon reunification. To learn what sensory modalities snapping shrimp use to identify their partner, we used a partner choice test where a snapping shrimp was allowed to freely interact with its partner and a stranger goby. Next, we altered the sensory abilities of *A. bellulus* by visually-blinding, chemo-blinding, or both, and repeated the partner choice test. Unaltered snapping shrimp spent more time contacting their partner compared to strangers. Snapping shrimp that were double-blinded spent similar amounts of time contacting both partners and strangers. We found a partial rescue of partner identification when antennae but not antennules were functional, suggesting antennae are used for chemical identification of partners. To ask if partner identity is encoded by antennal neurons, we recorded extracellular neural activity during interactions between snapping shrimp and their partner or a stranger. After FFT was applied to the data, we found higher neural signals in low and high frequency bands. This indicates that slow synaptic activity (low) and fast spiking activity (high) of neurons in the antennae are used to identify partners compared to strangers. When we compared band power before a snapping shrimp contacts its partner and during contact, we found the peak band power is significantly higher immediately before contact, indicating the importance of distance chemoreception. Taken together, we find that partner identification among mutualistic snapping shrimp is reliant on chemoreceptive input both before and during partner contact.

PS1.17

Motor systems, sensorimotor integration and behavior (Part 1)

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Environmental modulation of small-scale exploration in weakly electric fish

Animals actively explore their surroundings to efficiently obtain resources, relying on the sensory modalities available to them. In South American weakly electric fish, self-generated electric organ discharges (EODs) create a short-range ‘sensory bubble’ that, together with locomotion, supports exploration. This behavior relies on the coordinated modulation of spatial movement and electromotor activity, reflected in changes in EOD rate (EODr). However, how these components are engaged in natural contexts, and which conditions are sufficient to elicit exploration, remain poorly understood. Here, we examined how environmental and contextual factors modulate exploratory behavior in *Gymnotus omarorum* by combining a minimalistic laboratory assay with multi-day recordings in a

seminatural arena. In laboratory tanks, freely moving fish showed minimal locomotor activity, which increased only when darkness and a novel electrosensory stimulus co-occurred, indicating strong context dependence and gating by chronobiological and motivational factors. By contrast, under seminatural conditions preserving natural light and temperature cycles, fish exhibited robust nocturnal rhythms in both locomotor activity and EODr. Chronobiological analysis revealed that the electrosensory rhythm consistently preceded the locomotor rhythm, with individual phase differences correlated across behaviors. These results show that exploration is a temporally organized behavior arising from coordinated modulation of sensory and motor systems. By linking electrosensory activity, locomotion, and circadian timing under ecologically relevant conditions, this study provides insight into how animals regulate sensory sampling and movement during exploration.

PS3.91

Electrosensory systems

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Cellular transcriptomic evolution in the hindbrain of electric fishes (Gymnotiformes)

Evolution of novel physiological and behavioral phenotypes entails the evolution of neural circuitry to control it, with maybe none so enthralling as electrolocation. Several lineages of fishes independently evolved electric organs and electroreceptors for sensing their environment. The production and detection of these electrical signals are controlled by specialized brain regions. To understand what makes these neural systems unique requires examining how the cells composing these brain regions evolved. In South American gymnotiforms, the hindbrain contains both the first region that receives input from electroreceptors that detect electric fields, the electrosensory lateral line lobe(ELL), and the hindbrain region that controls their production by the electric organ, the pacemaker nucleus (Pn), both presumably evolved from nearby lateral line sensory regions and reticulospinal motor regions in non-electric fishes. Non-electric catfishes, such as channel catfish (*Ictalurus punctatus*), are an outgroup that has ampullary electroreceptors and a less differentiated ELL but no Pn, as they sense but do not generate electric fields. Likewise, the giant zebrafish (*Devario aequipinnatus*) has neither the ELL nor the Pn. These species represent a stepwise evolution of the neural mechanisms that regulate the detection and production of electric fields. Here we show how the cellular transcriptomes of the hindbrain evolved by comparing single-nucleus sequencing from giant zebrafish and channel catfish to two electric knifefish species, *Apteronotus albifrons* and *Brachyhypopomus gauderio*. The electric fish species had transcriptomically unique hindbrain neuronal cell types. We postulate that these cell types might correspond to neuron classes present in the ELL and Pn. Additionally, we explore the role of gene family expansion in the evolution of novel cell types. Taken together, our results demonstrate how transcriptomic evolution can lead to functionally novel cell phenotypes.

PS1.70

Spatial orientation and navigation (Part 1)

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A large-scale field dataset for studying navigation in the fiddler crab

Fiddler crabs (*Afruca tangeri*) are a burrow-centric species that navigate away from their burrows to engage in a variety of important survival behaviours, such as foraging and encountering potential mates. During such excursions, fiddler crabs continuously track their location relative to the burrow and remain vigilant for conspecifics that may take advantage of a vacant burrow. When threatened by a predator or a potential thief, they perform rapid beeline-type runs towards their burrow for safety or to defend it, even without a direct line of sight. This behaviour is suggested to rely on idiothetic information and local spatial cues, making them an attractive model organism for investigating how animals track their heading and position relative to a well-defined spatial reference point.
To perform a detailed quantitative analysis of the prevalence and diversity of these behaviours, we collected 27 hours of video data at the Ria Formosa estuary (Algarve, Portugal), between August and September 2023. Footage was captured during low tide and daylight hours, with two wide-field cameras covering an area of approximately 6 ◊ 3 m of the intertidal zone. Using a custom tracking model based on convolutional neural networks, we extracted the outward and homeward trajectories of approximately one hundred crabs per recording, and identified the spatial locations of their burrows.

The resulting dataset simultaneously captures individual and population-level navigation patterns and dynamics, allowing precise quantification of variables such as the direction and range of foraging areas, the straightness of homing trajectories, the extent to which individual crabs maintain burrow ownership and how these vary as a function of colony density. This large-scale analysis of navigation behaviours at the individual and collective level offers novel insights into the roaming patterns of fiddler crabs and how they navigate in relation to spatial landmarks and conspecifics in the wild.

PS3.53

Vision and photoreception (Part 2)

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What three-dimensional features are innately preferred across different insect pollinators?

The three-dimensional shape and complexity of flowers can limit how successfully insects forage from them. Since body plan and foraging strategies differ between insect pollinators- for instance, hawkmoths locate the nectary of a flower while hovering, while bees need to land to access the nectary of a flower - the preference for different shapes could differ across insect pollinators. The curvature of a flower is a key feature of the three-dimensional shape of a flower and impacts handling efficiency of insect pollinators . However, there are no studies that compare innate preference for curvature of floral objects across different insects. In this project, we perform cross-species investigations to compare the innate preference for different curvatures. We use *Bombus terrestris*, *Eristalis tenax* and *Macroglossum stellatarum* for this study because these species not only have comparable

visual systems, but are also known to use visual cues for foraging, and belong to different important pollinator groups - Hymenoptera, Diptera and Lepidoptera. We present individual pollinators with unrewarded, 3D printed artificial flowers of different curvatures and register their choices. By scoring the number of physical contacts on artificial flowers of different curvatures, we find that the bumblebees prefer bowl shaped flowers, while hummingbird hawkmoths prefer convex or flat flowers. These differences in innate preferences could arise because of their different foraging strategies. In further analysis, we will score the time pollinators spend contacting the different curvatures and visually inspecting them

PS2.56

Auditory systems and acoustic signaling (Part 2)

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Modelling active sensing collectives - can bats tracking consistency avoid jamming cost in groups?

Active-sensing animals, such as bats, probe their environment by emitting energy and analysing returning signals. Even a solitary bat's call generates a cascade of echoes from multiple objects, which the auditory system must segregate into one spatial scene. The problem of spatial scene reconstruction is exaggerated in social groups, where an individual must also discriminate its own echoes from the calls and echoes of conspecifics to prevent constant collisions. How, then, do bats navigate dense collectives without colliding even when facing intense jamming? We developed a biologically-parameterised dynamic model of active-sensing agents. We create an agent-based model and explicitly model bat behaviour and sound propagation. Inspired by the idea of optic and echo acoustic flow, we propose that bats may be detecting their own echoes in large swarms using spatio-temporal consistency. The echoes generated by obstacles around a bat are temporally locked to the bat's call emission. Hence sounds that are consistent, in terms of direction-of-arrival and delay post call, are more likely to be self-generated echoes. We demonstrate a dramatic reduction in group collision rates when individuals integrate information over larger time windows. Collision rates decrease by 29% on average when bats utilize consistency as compared with agents not using echolocation. Also agents utilizing spatio-temporal consistency works across an order of magnitude of group sizes (from 5-100 individuals) and show 30% lower wall collisions on average than the negative sensorimotor controllers simulated (non-echolocating agents). The behaviour of agents utilizing consistency-based controllers and agents with artificially perfect self-generated echo detection are remarkably similar. The behavioural similarity supports the idea that tracking consistent sounds in both direction of arrival and time delay improves echolocation under masking conditions. The modelling approach lays the groundwork to understand how multiple echolocators can show collective behaviour.

PS2.16

Motor systems, sensorimotor integration and behavior (Part 2)

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The neural basis of cuttlefish camouflage and social behavior

Cuttlefish are coleoid cephalopods that dynamically change the color, pattern and texture of their skin to camouflage with their surroundings. Camouflage is achieved by expanding

and contracting pigment-filled saccules in the skin called chromatophores, through the action of motor neurons that project from the brain. Thus, the patterning of the skin is a physical manifestation of neural activity in the brain. In addition to camouflage, cuttlefish use their dynamic skin to communicate with conspecifics using a series of innate skin patterns that reveal the animal's internal state. We are using the cuttlefish skin behaviors to understand how the physical properties of the visual world are represented by patterns of neural activity in the brain, and how this representation is transformed into an approximation of the physical world on the skin. We have performed a series of experiments to develop the dwarf cuttlefish, *Ascarosepion bandense*, as a model to investigate the neural basis of camouflage and social communication. We have described the stages of embryonic development, sequenced the genome and transcriptome, completed a 3D brain atlas, developed camouflage and social behavioral paradigms, and examined dwarf cuttlefish behavior in the wild. Furthermore, we recently succeeded in generating transgenic cuttlefish that express genetically-encoded calcium indicators in the brain, permitting the live imaging of neural activity. We are now using these technologies to simultaneously record neural activity and measure behavior to uncover how visual information is deconstructed in the brain, and then reconstructed into an image of the physical world on the skin.

PS3.50

Vision and photoreception (Part 2)

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Sensory drive selects for salience of courtship color and motion in *Habronattus* spiders

Sensory drive theory posits that sensory systems and associated signaling traits should co-evolve under selection for increased efficacy, such that evolutionary trajectories reflect adaptation to divergent signaling environments. Evidence for this process is well established in aquatic systems, but results from terrestrial taxa remain mixed. Using *Habronattus* jumping spiders, we tested the sensory-drive-based prediction that male courtship elements have evolved to be most salient in their own environments by 1) comparing color contrasts of focal species in their home environments to those across environments of all other species in our study, and 2) comparing parameters of courtship movements to motion characteristics of backgrounds in native habitats. For color ornaments, we find a consistent pattern of higher salience in home environments compared to heterospecific microhabitats. For courtship movement, we reveal a strong signature of habitat motion suggesting a history of selection for increased motion salience. Taken together, our work reveals a key role for sensory drive in the evolution of courtship elements in this terrestrial animal group.

PS3.64

Vision and photoreception (Part 2)

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Insights into the structure and function of dinoflagellates ocelloid

Dinoflagellates of the Warnowiidae family are single cell marine plankton that possess a complex camera-type eye structure: the ocelloid. They are also known to be fast swimmers, using two flagella to perform rapid movements. Little is known about the function of the ocelloid, but our hypothesis is that they are image forming eyes that allow them to visually detect and capture prey. We are interested in how they process visual information, and how it can be used to control their movement, in particular homing on their preys. To get a better understanding of dinoflagellates visual system and how it might connect to the swimming and hunting apparatus, we characterised their autofluorescence and performed immunostaining to map the expression patterns of actin and tubulin. Interestingly, we found high autofluorescence in the retinula. We also observed high density of tubulin in both flagella, and high density of actin in nematocysts. Furthermore, we are designing a custom-made behavioural setup that would allow us to record dinoflagellates movement in response to visual stimulation.

PS3.90

Electrosensory systems

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Catching sparks: habitat use and behavioural dynamics of African electric fish in Lake Nabugabo, Uganda

Electric organ discharges (EODs) grant electric fish special sensory abilities - and supply researchers with unique opportunities to record the behaviour of these animals in their habitats. In Lake Nabugabo, Uganda, the introduction of the predatory Nile perch in the 1950s has displaced many native species from the main lake. Hypoxic swamps and structurally complex habitats have served as refuge and contributed to a resurgence of the native fauna since then. Here, four mormyrid species inhabit a variety of habitats with steep contrasts in oxygen availability, temperature, flow, and structural complexity. Using continuous 24-hour multi-site recordings spanning the rainy season, we reconstructed species distribution and temporal activity patterns across a river inlet, the main lake, and a severely hypoxic wetland. We found EOD characteristics that point toward breeding activity - including elongated discharges consistent with hormonal masculinization and double-pulse signatures indicative of juvenile fish - as well as social signaling, evidenced by transient phase-locking between individuals. Our recordings also captured evidence for the presence of mormyrids in the open waters of the lake, and along the margins of floating papyrus islands, that were displaced into open water by flooding events. Two species appear largely excluded from the hypoxic wetland despite its value as a predation refuge, suggesting physiological tolerance actively shapes community distribution alongside predation pressure. In summary, these recordings document, how the electric fish of Lake Nabugabo have adjusted to survive in an ecosystem that exposes them to high predation pressure, harsh environmental conditions, and competition for resources.

PS1.4

Motor systems, sensorimotor integration and behavior (Part 1)

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Evolution and biomechanics of flight diversity across diptera
Flight performance in insects is shaped by physical scaling, aerodynamics, phylogenetic constraints, and species-specific selective pressures, yet their relative contributions to flight-motor evolution remain unclear. We quantified morphology in 133 Diptera species spanning the order's phylogeny and size range. For a subset of 46 species, we recorded hovering wingbeat kinematics using stereoscopic high-speed videography and modelled aerodynamics using full-DNS Computational Fluid Dynamics. Integrating these datasets in a phylogenetic context yielded three main results. First, wingbeat kinematics were highly conserved across most Diptera despite large morphological and ecological diversity, indicating strong aerodynamic constraints. Only two early-branched clades diverged: Culicomorpha (mosquitoes, midges) showed extreme high-frequency, low-amplitude wingbeats, whereas Tipulomorpha (crane flies) exhibited unusually low frequencies. Second, scaling analyses showed that Diptera maintain weight support across a three-order-of-magnitude mass range mainly through allometric increases in wingbeat frequency and relative wing size, while stroke amplitude remained size-invariant. Relative drag rose sharply below Reynolds numbers of 400, creating a viscous aerodynamic regime that elevates flight cost in tiny Diptera. Third, Culicomorpha displayed a pronounced aero-acoustic trade-off. Their high wingbeat frequency and enlarged flight musculature enhanced wingbeat-induced sound production used in swarm mate localization but simultaneously increased aerodynamic power demands relative to similarly sized Diptera. Together, these findings reveal how morphology, kinematics, and aerodynamics co-evolve under physical and ecological pressures. This comparative framework explains the strong conservation of wingbeat kinematics among Dipteran, and suggests that sexual selection may have driven Culicomorpha toward extreme flight strategies.

PS3.45

Vision and photoreception (Part 2)

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See you in the dark: molecular evolution of extreme visual systems in deep-sea fishes
Extreme environments provide natural experiments for understanding how visual systems evolve under strong sensory constraints. The deep ocean is among the most challenging visual habitats, characterized by extremely low light levels and a narrow blue-green spectral window, conditions that have driven remarkable diversification of visual systems in deep-sea fishes. Here, we investigate the molecular basis of dim-light vision using comparative genomics and transcriptomics, focusing on rhodopsin, the rod photopigment mediating scotopic vision. While most vertebrates possess a single rhodopsin gene, some deep-sea lineages exhibit extensive gene duplication; spinyfins (Diretmidae) represent an extreme case, with over 30 rod opsins spanning the blue-green spectrum. Several of these pigments show pronounced spectral shifts, including some of the shortest wavelength sensitivities known among vertebrate rhodopsins. Using a near chromosome-level genome assembly of the silver spinyfin, we show that rhodopsin genes are organized into two genomic clusters that are also functionally structured. Blue-shifted rhodopsins form a distinct cluster, exhibit the highest retinal expression, and display unique molecular features, including loss of the

conserved disulfide bridge (unique among vertebrates) and modifications to transducin-binding sites. Similarly, green-shifted variants are located in a separate cluster, suggesting coordinated evolution of gene duplication and genome architecture. To resolve how this molecular diversity translates into visual function, we use single-cell transcriptomics of the spinyfin retina to test whether multiple rhodopsins are co-expressed within individual rod photoreceptors or partitioned among distinct rod cell types, and to characterise rod photoreceptor identities. Together, our results demonstrate how gene duplication, molecular diversification, and photoreceptor cell specialization interact to expand visual capacity in extreme light environments.

PS3.12

Motor systems, sensorimotor integration and behavior (Part 3)

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Zebra finch vocal development beyond song

While vocal learning in juvenile zebra finches has been extensively studied by comparing juvenile and tutor songs, song represents only one component of their broader vocal repertoire. Zebra finches also produce a variety of calls, which include both tutor-learned and innate vocalizations. Examining both types offers a unique opportunity to compare distinct developmental processes and identify the mechanisms shaping vocal production. Here, we investigate the development of classical acoustic features and latent variables in the calls of juvenile male zebra finches. Furthermore, we aim to elucidate how these developmental trajectories are influenced by the social environment and access to tutor calls. Juvenile zebra finches were housed in sound-attenuated chambers from 30 to 90 days post-hatch, either raised in isolation or paired with an adult female. To ensure normal song learning, all birds received tutor song playbacks. We recorded the juveniles' daily vocalizations during exposure to isochronous 1 Hz call playbacks. To track call development, vocalizations were classified into short (unlearned tet/stack calls) and long (learned distance calls) types using UMAP and HDBSCAN clustering. We then quantified classical acoustic features and latent variables to characterize their developmental trajectories. We found that only a subset of birds developed characteristic frequency modulation (FM) in their distance calls during the critical learning period. Specifically, FM was, on average, higher in juveniles raised with a call tutor compared to those raised in isolation or with an adult female. Ultimately, this work provides a detailed, systematic framework for characterizing vocal development beyond song; by integrating quantitative approaches with controlled social manipulations, our study clarifies how social experience shapes the structural development of the complete avian vocal repertoire.

PS2.30

Vision and photoreception (Part 1)

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Morphological and behavioral evidence for sensory feedback in dynamic color change

Dermal photoreception has been well-evidenced in color-changing animals since chromatophores in isolated skin redistribute pigment in response to light. While the function of dermal photoreception in color-change behavior remains unknown, there are two leading hypotheses. The first hypothesis predicts that dermal photoreception acts as an environmental sensor, and the second suggests that it acts as a self-sensor, detecting the animal's current color state within a feedback system to optimize color change. Here, we tested the self-sensor hypothesis morphologically and behaviorally in the Caribbean hogfish (*Lachnolaimus maximus*), a color-changing fish that expresses a single visual opsin (SWS1) in cells underneath chromatophores. We hypothesized that these cells may detect differential light information due to the redistribution of pigment in overlying chromatophores, providing a self-assessment of color change. First, we evaluated whether the optics and morphology of the skin could support this hypothesized mechanism. We utilized microspectrophotometry to quantify light transmittance through chromatophores in aggregated versus dispersed states, and we calculated the spatial overlap between SWS1 cells and the two chromatophore states from confocal micrographs. We found that it is possible for underlying SWS1 cells to detect changes in pigment state based on the differential light transmittance and spatial overlap of aggregated versus dispersed chromatophores. Next, we conducted a behavioral experiment to test whether disruption of vision via blindfolding impaired color change. We found that blindfolding significantly impaired color-change responses, so dermal photoreception alone was not capable of mediating color change in the live fish. Together, these results lend support to the self-sensor hypothesis, suggesting that dermal photoreception may play a feedback role in coordinating color-change behavior as opposed to an environmental sensing role.

PS3.97

Methods

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Comparative effectiveness of iodine and phosphotungstic acid staining for micro-CT visualisation of the agamid brain

Non-invasive, three-dimensional (3D) bioimaging techniques, such as X-ray micro-computed tomography (micro-CT), have significantly advanced morphological and neurobiological research in recent decades. By using specific contrast-enhancing chemical stains, micro-CT can generate non-destructive, high-resolution, high-contrast images of animal soft tissues, enabling the visualisation of anatomical structures across multiple levels of biological organisation. We tested the effectiveness of inorganic iodine (I-KI) and phosphotungstic acid (PTA) as contrast-enhancing agents in aqueous solution for micro-CT visualisation of the nervous system in dragon lizards (Squamata: Agamidae). We identified significant differences between the two agents in terms of nervous tissue specificity, contrast quality, and levels of brain tissue shrinkage. Our results reaffirm inorganic iodine as the most comprehensive contrast agent for nervous tissue visualisation in reptiles using micro-CT, providing clearer differentiation between grey and white matter and reducing pH-related shrinkage artefacts. These findings highlight its potential to resolve neural structures within the brain and support its applicability to the study of vertebrate specimens from existing museum collections. Accordingly, we used diffusible iodine-based contrast-enhanced micro-computed tomography (diceCT), to investigate brain morphology in three agamid genera: *Ctenophorus*, *Diporiphora*, and *Tympanocryptis*. Australian agamids provide an excellent model for elucidating patterns of brain evolution and functional diversification due to their ecological diversity occupying both terrestrial and arboreal habitats. By quantifying the volume of major brain regions and using geometric morphometrics, we were able to test for associations between habitat use and mosaic brain evolution.

PS2.81

Learning, memory and cognition

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Measuring judgment biases in wild bumblebees

Judgment bias tests are used to evaluate affective states and provide insights into animal welfare. Stressed individuals typically make more pessimistic choices, and laboratory studies have demonstrated this effect in honey bees and bumblebees. However, the affective states of wild bees remain largely unexplored. We developed a mobile go/go active choice task with three ambiguous visual cues to assess wild bumblebee affective states in the field across habitats of varying stress (low, medium, high), defined by land-use intensity. Five bumblebee species were tested in the field. Bees successfully learned the task, and participation rates varied across species and locations. Our results also suggest some influence of land use on judgement biases. This method enables non-lethal assessment of stress in wild bees, and provides a novel framework for studying welfare in free-living pollinators, and a useful method to assess the cumulative effects of different stressors on bumblebees in natural environments.

PS3.40

Social behavior and neuromodulation (Part 2)

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The effects of sleep disruption and temperature on shoaling cohesion in the Trinidadian guppy (*Paracheirodon axelrodi*)

Sleep is a fundamental neurophysiological and behavioural state, observed ubiquitously across the animal kingdom. While the function of sleep remains elusive, its importance is evident through the deleterious effects of sleep deprivation. In fish- an understudied taxon in the context of sleep behaviour and ecophysiology- sleep disruption has been linked to impaired cognition, altered movement, and reduced predator avoidance responses. As ectotherms, fish are particularly vulnerable to climate change, with increased temperatures associated with a plethora of behavioural and physiological changes. However, the impact of climate-related stressors on sleep in shoaling and group behaviour is largely unexplored. The present study aimed to address this gap by investigating 1) how varying degrees of sleep disruption (one night or three nights) affected group cohesion, and 2) whether temperature modulated the effect of sleep disruption on group behaviour. Using groups of 5 Trinidadian guppies (*Poecilia reticulata*), we implemented a repeated measures experimental design with four disruption treatments- one versus three days of intermittent physical disruption in the day and the night- at two temperatures (23 and 29). To assess how different degrees of sleep disruption, alongside temperature differences, affected groups of guppies, we used an open field test to measure movement, shoal cohesion and polarity after each disruption treatment, and then quantified behavioural changes using video tracking software. This study represents a novel investigation into how sleep disruptions, alongside temperature change, may disrupt shoaling activity and efficiency, with potential implications for predator avoidance and energy expenditure.

PS3.89

Electrosensory systems

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Action potential energetics in high-frequency weakly electric fish

The processes underlying neuronal action potentials constitute a relatively high fraction of nervous system energy budgets. Most of it is due to the activity of the sodium-potassium pump (Na+-K+ ATPase) to maintain the ion gradients required for action potential generation. Sodium (Na+) and potassium (K+) currents that underlie an action potential flow in opposite directions and often overlap in time, leading to effectively 'wasted' current and unnecessary activity of the Na+-K+ ATPase. Minimizing this overlap can thus be a strategy for energy efficient neural computation. The black ghost knifefish, a species of weakly electric fish, uses a quasi-sinusoidal electric organ discharge (EOD) to navigate, capture prey and communicate. Brainstem pacemaker neurons control the EOD in a 1:1 fashion at frequencies over1000 Hz. We hypothesize that the Na+ and K+ currents of pacemaker neuron action potentials are optimized in time to reduce the high energetic demands associated with sustained high-frequency firing. Using a broad parameter survey of a biophysically based computational model of a pacemaker neuron, we found that the energetic cost of an action potential was especially sensitive to parameters related to Na+-channel gating. These findings suggest that the rapid evolution of voltage-gated sodium channels in these high-frequency fish may have been driven, at least in part, by the energetic constraints of action potential generation.

PS3.59

Vision and photoreception (Part 2)

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Negative phototaxis in juvenile sea stars (Class Asteroidea)

It has been noted in the literature for over a century that sea stars (Phylum Echinodermata, Class Asteroidea) have eyes, yet thorough investigations of the structure of their eyes, vision, and visual ecology are relatively recent. As keystone predators in benthic habitats worldwide, sea stars play important roles in shaping the structure of marine communities. In addition, understanding their behavioral and visual ecology at all life stages is crucial, as the juveniles of some species (i.e. *Pycnopodia helianthoides*) have been observed to eat 10 times as many prey items per day as adults. Previous work on the Crown-of-Thorns Star (*Acanthaster planci*) demonstrated that juveniles exhibit negative phototaxis yet are unable to orient to visual stimuli, likely because their eyes are unable to perform optically at their

small size. Other sea star species remain untested. Here, we present an investigation into the eyes and visual responses of asteroid species from the Salish Sea (Pacific coast of the northwestern United States). Consistent with previous results, juveniles of the species *Solaster stimpsoni* and *Leptasterias hexactis* exhibit negative phototaxis and do not orient to a visual stimulus.

PS1.19

Motor systems, sensorimotor integration and behavior (Part 1)

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Distinct roles of giant interneurons in wind-elicited escape behavior

Escape behaviors are crucial for animal survival and are conserved across a wide range of vertebrate and invertebrate species. Escape behaviors require the rapid transformation of sensory information into motor commands to control speed, distance, and direction of movements. Previous studies on Mauthner cells in fish and Giant fibers in crayfish have provided key insights into descending motor circuits that control the escape movements. However, the behavioral functions of ascending sensory interneurons in transforming sensory information about stimulus intensity and orientation into motor commands for escape behavior remain unclear. To address this issue, we focused on the wind-elicited escape behavior in crickets because identified giant interneurons (GIs) are assumed to provide the ascending signals correlated with that behavior. We investigated the causal relationships between GIs and escape behavior by combining a single-cell photoablation with simultaneous recordings of GI's activity and escape movement. We selectively ablated a specific GI and monitored the wind-elicited behavior before and after the ablation. Ablation of the GI 8-1 reduced response probability and prolonged reaction time in the escape response to airflow stimuli from the back. In contrast, ablation of GI 10-2 significantly increased the dispersion of escape directions in response to back stimulation, without suppressing escape initiation. To further examine direction-dependent effects, we applied airflow stimulus from three directions: the posterior of the cricket and the ipsilateral and contralateral sides to the soma of the targeted GI. GI 8-1 ablation consistently reduced response probability and delayed escape initiation across stimulus directions. In contrast, escape direction remained unaffected by GI 8-1 ablation. Together, these results revealed that individual GIs play distinct roles in wind-elicited escape behavior.

PS1.57

Social behavior and neuromodulation (Part 1)

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Neural representation of sound direction underlying phonotaxis in *Aedes aegypti*

Identifying the sound source is sometimes critical for successful acoustic communication. Males of the yellow fever mosquito, *Aedes aegypti*, find their potential mates within a complex swarm by using their sensitive auditory system. Male mosquitoes are phonotactically attracted to females' wingbeats by . The mechanism of sound source localization has long been studied in animals with tympanal auditory systems since Lord Rayleigh proposed the binaural effect. On the other hand, because the antennal system used by mosquitoes is fundamentally different from the tympanal system, previous studies have proposed distinct models, including the possibility that sound direction is encoded in one ear. However, how auditory direction was processed in the mosquito brain remains unknown. A mosquito antennal ear is composed of a flagellum that vibrates in response to sound and a pedicel comprising Johnston's Organ (JO) neurons that form a ring around the flagellum. As the flagellum vibrates in any direction, previous studies proposed that a distinct population of JO neurons responds to the different directions of sound, projecting excitatory input to the AMMC, perhaps in a directionally-selective map. To test this, we performed calcium imaging in the auditory brain regions of transgenic mosquitoes expressing GCaMP6s, used dynamical system models, and imaged the velocity field around the antennal flagellum using Particle Image Velocimetry, thereby providing a physical explanation for the directional properties of JO neurons. From the calcium imaging experiments, we observed calcium responses of auditory sensory neurons, JO neurons, in the brain while delivering a series of pure tones from distinct directions. The direction preference of male JO neurons was diverse across individuals, while female JO neurons responded significantly stronger to the contralateral sound source than the ipsilateral. We also analyzed the spatial distribution of direction preferences in JO neurons from a single antenna. The directional responses were diverse across AMMC loci, and were not localized in a directionally-selective map. This indicates that the interaural intensity difference is not generated by the same mechanism as in tympanal hearing systems.

PS1.22

Motor systems, sensorimotor integration and behavior (Part 1)

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An ethological assay using prey-capture and escape to investigate goal-switching in mice

Hunting for prey and escaping from predators are core instinctive behaviours whose appropriate switching is crucial for survival and requires accurate orientation toward the respective goals: prey or shelter. In vertebrates, the Superior Colliculus (SC) is a key neural circuit that controls orientation to targets and is necessary for both hunting and escape behaviour. We are investigating the hypothesis that the SC governs goal selection during hunt-to-escape switching by competitively encoding the current target and using this representation to guide orientating actions toward it. To achieve this, we developed a paradigm where freely behaving mice switch from hunting to escape by interrupting cricket pursuits with threatening looming stimuli that trigger shelter-directed escapes. We find that mice break hunting routines by rapidly escaping from the threat, and that the choice between pursuit and escape is probabilistic, suggesting an ongoing competition between prey and shelter goals. To investigate this switching process under rigorous stimulus

control conditions we then developed a head-fixed virtual reality (VR) setup. Mice move their head in yaw and are surrounded by four monitors displaying a VR environment, within which they chase crickets tethered to a motor-controlled arm responding to their movements. Mice engage in hunting pursuits by orienting and running towards the arm. During arm-chasing bouts, presentation of threatening stimuli elicits escape responses, with increased locomotion and sharp head-movements away from the arm. Using these two paradigms we are currently recording single-unit activity in the SC using multi-shank Neuropixels probes, identifying units tuned to cricket- and shelter-bound spatial variables and characterizing their dynamics during goal switching. Together, this work introduces a new assay for studying natural goal-switching and positions the SC as a key circuit for arbitrating competing survival drives in mice.

PS2.27

Vision and photoreception (Part 1)

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A locust neurovisual model processes complex stimuli via diverse interacting mechanisms

In locusts, a retinotopically mapped collision-sensitive visual pathway in the brain converges onto the Lobula Giant Movement Detector (LGMD), which is preferentially tuned to looming stimuli. Previous findings suggest that the LGMD is less sensitive to translating objects, wide-field motion, and incoherent images of looming objects. Experimental and modelling work have both shed light on the various mechanisms underpinning these properties. However, broad examinations of how these mechanisms interact with each other and with diverse stimuli have been limited. To address this, a computational model of a looming-sensitive circuit in locusts was developed. This model incorporated a diverse selection of mechanisms from recent biological literature and was subjected to an array of looming stimuli with varying size-to-speed ratio, polarity (OFF and ON), and coherence, with wide-field background motion, translating stimuli, and trajectory changes also being examined. The model shows quantitative and qualitative fidelity to biological data in its replication of a wide variety of stimulus responses, with its preference for incoherent visual stimuli over coherent ones being a notable exception. Based on investigations with various inhibitory mechanisms removed, it was shown that lateral inhibition predominantly suppressed wide-field motion responses. Global inhibition both normalized growing excitation over the course of looming, and improved recognition of looming stimuli against moving backgrounds. Diverse roles were observed for feedforward inhibition, including shaping peak response time and its variability, and improving coherence selectivity. The success of the model in replicating multiple stimuli also showed the plausibility of several underlying assumptions, including a sharing of input circuitry between the LGMD and multiple other neuron types which suggests a parsimonious visual motion detection circuit.

PS1.66

Spatial orientation and navigation (Part 1)

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Can goldfish learn to navigate a complex labyrinth environment?
Navigation is a complex cognitive behavior since it relies on multiple sensory and cognitive processes. To navigate, the animal must perceive its environment, the position of cues and obstacles in it, to comprehend its own position and the target position, as well as to plan possible routes. Fish, like most animals, explore complex environments rich with sensory cues that facilitate navigation. However, environments usually used in the lab to explore this behavior tend to be simple and basic. Therefore, there is a substantial gap between lab environments and natural environments. We propose to overcome this gap using a two-dimensional complex labyrinth composed of a 7-by-7 grid of interconnected cells which enables challenging navigation tasks. We found that goldfish can learn to navigate this complex environment when environmental cues are available. Fish were also able to learn the task in the absence of cues, however the learning was less efficient, indicating that environmental cues facilitate learning to navigate in complex settings. To further characterize the underlying navigational strategies, we compared goldfish performance to that of simulated walker-agent models implementing different navigational strategies. Goldfish outperformed all pure strategies, as well as combinations of two pure strategies, yet fish performance remained inferior to that of a full cognitive-map model. We applied a series of environmental manipulations that challenge goldfish' knowledge of the labyrinth and found that while environmental cues assist navigating in different settings, goldfish rely more on global beacon than on local landmarks. In addition, change in start position in the labyrinth significantly prolonged fish trajectory, which supports the notion that goldfish does not rely on full cognitive map representation to navigate the complex labyrinth. Overall, our findings contribute to the understanding of fish navigation in complex environments.

PS2.10

Motor systems, sensorimotor integration and behavior (Part 2)

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Artificial light at night alters flight trajectories of insects in the field
Artificial light at night (ALAN) has strong impacts on the behavior of flying insects, often producing characteristic flight patterns such as spiraling, stalling, or orbiting that can lead to entrapment near light sources. It is not clear whether insects actively fly toward artificial light or whether entrapment emerges from altered flight dynamics once individuals enter the illuminated region, nor at which distance insects become entrapped by light. To address these questions, we conducted high-speed video recordings of nocturnal insects at a field site in Costa Rica. Using two synchronized cameras positioned above a light source, we recorded short video sequences at regular intervals throughout the night. The light alternated between on and off conditions, allowing us to compare insect abundance and flight behavior in illuminated and dark conditions. From reconstructed trajectories we quantified features of their flight trajectories including path straightness and turning angles with respect to the light source under light-on and light-off conditions. We found that more insects flew through the field of view when the light was on, suggesting that ALAN is entrapping insects near the light source. Flight paths were more straight for insects flying through the field of view when the light was off, consistent with the dorsal light response to ALAN seen in flying insects. Understanding the spatial scale and behavioral drivers of light-induced entrapment will improve predictions of ecological impacts of ALAN and inform strategies for reducing its effects on nocturnal insect communities.

PS1.12

Motor systems, sensorimotor integration and behavior (Part 1)

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Multimodal VR system probes sensorimotor integration in locomotion–navigation coupling

Insects exhibit remarkable adaptive behavior in complex environments despite operating with compact nervous systems several orders of magnitude smaller than those of mammals. While locomotion has long been central to motor control research, adaptive behavior critically depends on goal-directed navigation-deciding where and why to move. Stick insects are a key model for walking neural control, yet their behavior extends beyond rhythmic locomotion to active exploration and spatial decision-making through multimodal sensory integration. They integrate antennal tactile sensing, visual orientation cues, and chemical signals such as plant odors or pheromones to guide navigation. Recent studies reveal rapid sensorimotor transformations, including tactile-to-reach conversion within ~40 ms and visually guided directional selection shaped by environmental structure and luminance. These findings point to tight coupling between locomotion and navigation through dynamic sensorimotor integration. However, how visual, olfactory, and tactile signals collectively shape motor output during navigation remains unclear under controlled, reproducible conditions. To address this gap, we developed a multimodal virtual reality (VR) platform for stick insects that enables real-time manipulation of visual, olfactory, and tactile inputs during locomotion. The system employs a spherical treadmill allowing tethered insects to walk freely while high-resolution kinematics and walking trajectories are recorded. Visual scenes are updated according to the animal's movement, enabling closed-loop analysis of locomotion-navigation coupling. This platform enables quantitative evaluation of how defined sensory contexts modulate walking dynamics, turning, and goal-directed behavior. By establishing reproducible multisensory conditions, it provides a framework to dissect neural principles of sensorimotor integration in navigation and clarifies how compact nervous systems generate adaptive behavior.

PS3.75

Olfaction, taste and chemical sensing

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A trigeminal-hindbrain circuit initiates a persistent chemosensory state in zebrafish

The ability to identify and respond to threats in one's environment is necessary for animal survival. The presence of dead conspecifics is a salient indication of danger, and animals

will persistently alter their behavior upon exposure to chemicals associated with death and tissue decay. The neural mechanisms producing these persistent innate behaviors are poorly understood. Here we describe a chemosensory assay for tethered zebrafish larvae, where fish experience a brief exposure to the death-associated odorant cadaverine. We found that exposure to cadaverine for one minute induces an elevated heart rate and suppression of visually-driven locomotion for 15 minutes. These effects are concentration-dependent, and specific to death/putrefaction-associated chemicals, but not unrelated chemosensory threats (ie: NaCl). Using brain-wide cellular-level calcium imaging, we found that brief cadaverine exposure induces long-lasting activity in the forebrain and hindbrain - including spatially clustered cells in the dorsolateral hindbrain and widespread recruitment of GABAergic neurons. Chemogenetic ablation of olfactory neurons suppressed cadaverine-driven activity in the forebrain; however, this manipulation did not prevent persistent tachycardia, locomotor suppression, or hindbrain activity, suggesting an alternate pathway for chemosensory detection of cadaverine. We found that the basic pH of high-concentration cadaverine solutions is necessary and sufficient to induce persistent tachycardia, locomotor suppression, and hindbrain activity. Imaging experiments indicate that neurons in the trigeminal ganglion - with sensory input from the face and axonal output to the lateral hindbrain - are responsive to high-pH cadaverine. Together, these findings demonstrate how diverse chemosensory systems detect environmental stimuli to mediate persistent survival behaviors.

PS3.26

Social behavior and neuromodulation (Part 2)

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Consistent personalities, surprising defenses: behavior across life stages and contexts in a tree frog

Amphibians are key ecosystem indicators and are highly sensitive to environmental change, making them powerful models for understanding how behavior responds to ecological variation. However, behavioral data is still scarce for many arboreal species, limiting our ability to link individual behavior to ecological and evolutionary processes. Here, we characterize behavioral variation in the fringed leaf frog (*Cruziohyla craspedopus*), integrating personality traits and antipredator responses across developmental stages and ecological contexts. We quantified activity, exploration, and boldness using behavioral assays conducted under both laboratory and field conditions. Behavioral variation was structured along two primary axes: an activity axis associated with locomotion and exploration, and a boldness axis associated with emergence responses. These axes were independent, indicating a multidimensional organization of behavior. We detected clear ontogenetic differences, with juveniles showing lower activity and greater use of ground substrates, whereas adults exhibited higher activity and increased use of vertical structures. Field-collected individuals were also more active and emerged more rapidly than captive-raised individuals, highlighting the role of environmental experience. In addition to consistent individual differences in routine behavior, we observed context-dependent defensive responses. Individuals displayed a previously undescribed ‘twerking’ posture under laboratory conditions, along with stress-induced color changes and the release of volatile compounds, suggesting multimodal antipredator strategies. Together, our findings

reveal that both personality traits and defensive behaviors are shaped by life stage and environment, providing new insights into how behavioral strategies are organized and expressed in arboreal amphibians.

PS2.3

Motor systems, sensorimotor integration and behavior (Part 2)

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Perineuronal nets in vocal nucleus HVC regulate developmental song plasticity in finches

Many communicative behaviors, including human speech and birdsong, are learned during critical periods in development and play important roles in social behavior, survival, and reproduction. Thus, understanding the neural mechanisms shaping the acquisition and development of communication is crucial. Perineuronal nets (PNNs) are extracellular matrices that surround neurons and modulate plasticity, including the neurons in sensorimotor circuits regulating vocal motor learning and performance in songbirds (the 'song system'). PNNs have been implicated in the closure of developmental critical periods, where their maturation is thought to stabilize circuit structure and to limit plasticity. In the song system, PNN expression increases over development, and degrading PNNs in the vocal motor nucleus HVC affects adult song performance. However, little is known about how manipulating PNNs earlier in development influences song development and learning. To this end, we bilaterally degraded PNNs in the HVC of juvenile male zebra finches (<60 days post-hatch) and tested the hypothesis that degrading PNNs would (a) increase the rate or extent of natural song development and (b) enhance the ability of juveniles to learn novel song elements. In our analysis of natural song development, we found that songs changed significantly more quickly following PNN degradation, though not necessarily towards the tutor's (father's) song. Our preliminary analyses also indicate that PNN degradation allows juveniles that were raised and tutored by their father to learn new song elements from a novel tutor (re-tutoring). These data highlight a causal role of PNNs in vocal motor circuitry in regulating song learning and development and support their potential role in critical period regulation.

PS2.96

Learning, memory and cognition

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Considering learning as a categorical variable

Learning has long been a topic of interest in honey bee neuroethology. Evaluation of learning of individual subjects is a topic that has recently begun to gain more traction both within honey bee research and within neuroethology as a whole. Learning is considered observable as a sigmoidal process, whereby the performance of an individual in a behavioural assay theoretically increases from chance level to a plateau near 100%. As a result, logistic regressions are a popular method for evaluating learning, where the slope represents the strength of learning. This allows for the evaluation of learning on both the level of individual subjects and the entire sample while also adding more nuance to the

performance results. However, in more complex experimental setups where learning is a prerequisite to progression to a second stage of experimentation, this evaluation method makes it difficult to assign subjects as having learned or not learned due to the continuous nature of its output. Using a combination of change point analysis and Bayesian statistics can both allow for the benefits of a logistic regression while also allowing for easy categorization of subjects as having learned or not learned. This can be accomplished without sacrificing the ability to treat overall performance of the subjects as a continuous variable. Further, Bayesian statistics is robust to small sample sizes and starting position for the analysis. This project evaluates the use of these methods in analyzing a simple honey bee binary choice operant conditioning assay using a y-maze and examines both its use in evaluating binary results as well as the effectiveness of using its output to evaluate behaviour within the maze.

PS3.48

Vision and photoreception (Part 2)

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Temperature-compensated tuning to motion in putative neurons of the central complex

Bumblebees use path-integration to find the shortest path to their nest. This requires tracking and integration of heading angle and distance travelled. Flying bees estimate flown distance based on translational optic flow. The central complex (CX) is believed to be the neuronal substrate for path integration in insects. It receives information about translational movement from tangential neurons of the noduli (TN neurons, LNO neurons in *Drosophila*) that are sensitive to optic flow. While neuronal processes are typically temperature-dependent, it would be beneficial if encoding of distance was independent of temperature. Bumblebees keep their body temperature constant within certain limits of the ambient temperature, but when the ambient temperature exceeds 25 ∞C the bee's temperature rises as well. We therefore asked if the encoding of translational optic flow in TN-like neurons is independent of head temperature. To test this, we employed extracellular tetrode recordings. Bumblebees were fixed in the center of an LED-arena consisting of 16x128 green LEDs covering 45∞x360∞ of visual space. Translational optic flow was presented as progressing and regressing stripe patterns (spatial frequency: 0.022 cycles/∞, velocity: 14 ∞/s-2812 ∞/s). Velocity response curves were measured at two head temperatures (23 ∞C and 32 ∞C). Neurons were identified as TN-like, based on their response properties and the recording location. Our preliminary results from 4 TN1-like units and 5 TN2-like units show that these neurons did not shift their preferred stimulus velocity when temperature changed. This is different from most motion-sensitive neurons outside the CX. Furthermore 4 out of 5 TN2-like units that responded excitatory to front-to-back optic flow (simulated forward flight) did not change their overall activity with temperature and therefore seem to be fully temperature-compensated. This could facilitate robust distance estimation, independent of ambient temperature.

PS1.81

Evolution and development

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Towards the anatomical blueprint of the insect brain

The insect brain displays highly similar anatomical patterns with clearly homologous regions across all insect groups, despite having diverged from a common arthropod ancestor more than 450 million years ago. Distinct regions can be assigned to primary sensory processing (visual and olfactory), to long term memory, action selection and motor control. The central complex (CX), the only unpaired region of the insect brain, is responsible for context dependent action selection, in the context of spatial navigation and orientation tasks. For decades, the CX has been identified and studied with traditional histological methods in many insect groups. In lepidopteran brains, we established that the components of the CX are amongst the least variable brain regions across species, forming a tightly linked group of neuropils, with little room, or need, for substantial modifications during evolution. Particularly in larger species, the CX occupied smaller relative volumes, indicating diminishing returns from investing in this region beyond a certain required size. This was contrasted by other brain regions that showed larger evolvability, in line with ecological demands. It remains unknown how these findings translate to a wider range of species, and how, in general, phylogeny and ecological needs constrain the evolvability of the CX, its associated neuropils, as well as components of its input and output pathways. To understand how these regions have evolved in the context of the insect brain, we therefore aim at establishing detailed anatomical reconstructions of the CX and its surrounding brain regions across a wide range of insect species. Using micro-CT we imaged whole insect heads, segmented brain neuropils, and extracted their volumes. Based on data from across the insect phylogenetic tree, and covering a wide range of ecologies, we describe the detailed anatomical layout of insect brains, from the primitive Zygentoma and damselflies to bees, beetles and hoverflies.

PS1.45

Social behavior and neuromodulation (Part 1)

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Aggression and season shape circulating fatty acid profile of wild song sparrows

Territorial aggression is critical for resource and food acquisition but imposes substantial metabolic costs. Meeting these energetic demands requires the mobilisation of fatty acids (FAs) from lipid stores to fuel intense activity. Circulating FA profiles therefore reflect critical substrates to sustain aggressive behaviours. Because FAs are derived from diet, seasonal variation in food availability and territory-mediated food access may also shape FA profiles. Consequently, a bidirectional relationship may exist in which aggression and season shape circulating FA profiles, while FA availability may in turn modulate aggression. Here, we investigated the impact of an aggressive encounter on circulating FA profiles in wild non-migratory male song sparrows (*Melospiza melodia*) across seasons. Plasma was collected from birds exposed to either a control condition or a 10-min simulated territorial intrusion (to induce aggression) in the breeding and non-breeding seasons (n=12/season/condition). Following lipid extraction, FAs were derivatised via acid-catalysed methylation with acetyl chloride, which hydrolyses complex lipids to release total FA for analysis. We then quantified 34 medium-chain and long-chain FAs using gas chromatography flame ionisation detection. Ongoing analyses will characterise short-chain FAs. We found that both aggression and breeding season were associated with global decreases in circulating FA concentrations. Moreover, 9 FAs varied with aggression while 20 varied with season. Notably, these two factors interacted, where aggression increased the average FA chain length in the non-breeding season but decreased it in the breeding season. These findings suggest that seasonal physiological demands reshape metabolic responses to social conflict.

PS1.24

Motor systems, sensorimotor integration and behavior (Part 1)

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A parametric guidance law model of hunting in mice

Mice rely on eating highly motile insects in the wild. Despite extensive work on hunting in mice, a basic description of hunting pursuit has not been made. Here we describe hunting pursuit as a parametric guidance law model, and relate this guidance law to multiunit brain activity. We found that of two common guidance laws, parallel navigation and proportional pursuit, parallel navigation better fit the behavior, though the difference was not statistically significant. How mice implement such a strategy necessitated an investigation into how behavioral variables relevant to hunting were represented in the brain. We investigated the superior colliculus (SC) due to its dual role as sensory and motor region. We found rich representations of the hunt in SC through multilayer Neuropixel probe recordings. Linear decoding yielded accurate reconstructions of such variables as distance, ground speed and turning speed in a state-dependent manner. These results indicate SC as a promising avenue for understanding complex and naturalistic sensorimotor behaviors such as hunting, and that there are state-dependent representational shifts in SC.

PS3.61

Vision and photoreception (Part 2)

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Evolutionary patterns of arthropod opsin evolution and color vision

Arthropods were the dominant animal group during the Cambrian Explosion, as well as the first animal group to invade terrestrial habitats, offering an exceptional opportunity for understanding the evolution of color vision from the earliest appearance of animal eyes. We identify the major themes of the molecular evolution behind arthropod color vision, integrated across biological scales and environmental variables by assembling over 5270 opsin sequences and associating them with photoreceptor expression patterns, spectral data and species ecologies. In evolutionary analyses, arthropod opsins continue to cluster into three main phylogenetic clades that loosely correspond to sensitivity in three overlapping portions of the visible spectrum. Arthropods predominantly express opsins found in the 'long-wavelength-sensitive' clade, with opsins in the 'middle-wavelength clade' found primarily in aquatic crustaceans. Although ecological patterns are driven by the order

of magnitude more data available from insects, analyses found significant patterns across activity cycles, where diurnal species express more opsins and have more retinal spectral sensitivity peaks than nocturnal species, and predatory species expressed more opsins than non-predatory species. Unexpectedly, in analyses of habitat medium, aquatic species expressed significantly more visual opsin transcripts than retinal spectral sensitivity peaks while terrestrial species had significantly more spectral sensitivity peaks than expressed opsins across their retinas. These analyses outline the large gaps in knowledge about visual ecologies across this group, and highlight the association between the expression of large numbers of opsins and aquatic habitats, a pattern that is not yet well understood.

PS3.36

Social behavior and neuromodulation (Part 2)

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Environmental and neuroendocrine drivers of vocal behavior in a South American frog

Understanding how individuals communicate and engage socially offers a framework for explaining the emergence of collective behaviors, from brain-level activity to communication patterns and dynamic social exchanges. Some anuran species are known for forming choruses and producing collective vocalizations as a strategy to attract females during courtship. Chorusing has been studied for its adaptability to environmental conditions, particularly in tropical populations. Nevertheless, our understanding of the neuromodulatory mechanisms that shape individual vocal output remains limited. *Boana pulchella* is a subtropical hylid anuran native to southern South America, characterized by a long breeding period. During courtship nights, males form choruses and emit three distinct types of vocal notes. Our research integrates field studies at two scales: (1) population-level passive acoustic monitoring to assess annual chorusing activity and its relationship with environmental variables, and (2) individual-level recordings and pharmacological field trials to examine how neuromodulation and social context influence vocal behavior. We show that choral activity is strongly associated with environmental drivers, particularly photoperiod and temperature. At the individual scale, males adjust their vocal output according to social context, and we identify a neuromodulator involved in call production. Together, these findings advance our understanding how collective behavior arises from the interplay between environmental conditions, social dynamics, and neuroendocrine mechanisms.

PS1.5

Motor systems, sensorimotor integration and behavior (Part 1)

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A state-dependent neural circuit resolves approach–avoidance conflicts

To survive, animals must balance opportunity and risk, yet how internal motivational state biases this trade-off remains poorly understood. Here we show that hunger gates nociceptive avoidance in *Drosophila* to enable food approach under conflict. Using a

behavioural assay in which flies must cross an electric shock barrier to reach food, we find that starvation promotes shock tolerance only when food-related olfactory cues are present. This state- and context-dependent behavioural shift is mediated by a defined neuromodulatory circuit. Leucokin in neurons encode hunger and convey this information to PV5K1 lateral horn output neurons, which integrate internal state signals with food odour cues and suppress shock-responsive FB2B neurons in the ventral fan-shaped body. Accordingly, electric shock-evoked activity in FB2B neurons is suppressed in starved flies exposed to food cues. These findings identify a circuit mechanism by which motivational state gates aversive processing to bias action selection towards goal-directed behaviour.

PS3.13

Motor systems, sensorimotor integration and behavior (Part 3)

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Adaptive winner-takes-all motor competition in dueling neural networks

How do motor control networks which generate divergent, mutually-exclusive outputs dynamically interact to ensure appropriate and smooth locomotory transitions? While central pattern generators (CPGs) are well known to produce rhythmic motor output, less is known about how circuit dynamics shape the timing and structure of transitions amongst competing CPG networks. Using calcium imaging of fictive locomotion in the *Drosophila* larval locomotor system, we explored the dynamics of switching between distinct locomotor programs to interrogate mechanisms of neural resilience and motor competition. Here, we provide evidence that *Drosophila* show 'since-last-state' memory and follow patterns of adaptive, 'winner-takes-all' dynamics. We found that transitions amongst fictive motor programs are non-random, with anterior asymmetric fictive patterns - the neural correlate of exploratory head sweeping behaviour in intact animals - operating as a transition hub towards more diverse, variable outputs. Further, we show that transition dynamics amongst motor programs are best modelled through time-since-last-state memory whereby transitions are biased towards motor programs that have occurred least over time. Moreover, by examining effects of octopaminergic neuromodulation [1], we reinforce the notion that anterior asymmetric fictive programs appear central to ensuring motor diversity after a period of sustained, constrained fictive output. Overall, we propose a new way of conceptualising interactions amongst motor programs: competing, functionally distinct CPGs can be engaged in adaptive, winner-takes-all competition to ensure maximal diversity of potential fictive output over time. Finally, as a Laboratory Efficiency Accredited Framework (LEAF) Gold accredited group, we have estimated the CO2 equivalent emissions associated with our work using WillCO2st, a platform designed to track carbon intensity of research activities [2, 3]. [1] Smith & Pulver, 2025 10.1101/2025.08.17.670699 [2] - Smith et al., (2026) 10.1093/genetics/iyaf268 [3] - Smith et al., (2026) 10.64898/2026.01.30.702771

PS2.8

Motor systems, sensorimotor integration and behavior (Part 2)

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Deciphering the neural pathway responsible for the initiation and progression of introductory notes in female-directed song of the zebra finch

Male zebra finch song is a key component of courtship behavior directed towards a female, making it a good example of a naturally learned motor sequence. Song bouts typically begin with a series of introductory notes (INs) before the song is repeated multiple times. As INs repeat, the intervals between successive notes decrease, and their acoustic properties progressively change, reaching a consistent state just before each song. Previous lesion studies have shown that components of the song motor pathway, including HVC, RA, and the dopaminergic nucleus A11, are critical for initiating female-directed song. However, their specific roles in the initiation and progression of INs remain unclear. Here, we investigated the role of HVC in the initiation and progression of INs using experimental manipulations of HVC in combination with a controlled experimental paradigm in which a smart film precisely regulated the timing of female presentation. In the paradigm, birds reliably initiated INs and songs following female presentation (86.93 ± 2.93% of trials). The first IN occurred at 821.08 ± 50.08 ms, and the first song at 2624.52 ± 120.81 ms. As described earlier, birds with electrolytic lesions of HVC did not produce song. However, we found that they produced short vocalizations that mostly resembled INs and calls (non-song vocalizations). While the latency to initiate the first IN and the total number of INs remained largely unchanged, intervals between successive INs were longer, when compared to sham lesions. To further probe HVC's function, we electrically stimulated HVC during INs. Preliminary results (n = 2) showed that electrical stimulation of HVC with a 400Hz train of pulses for 2s, immediately following female presentation, increased the time to the first IN and reduced the proportion of trials with song. Overall, these data suggest that HVC activity controls the timing of INs and is partially involved in the control of initiation of INs.

PS2.103

Education

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A conceptual framework for virtual reality simulation in undergraduate nursing: enhancing early recognition of clinical deterioration

Aim - To develop a conceptual framework for virtual reality simulation in undergraduate nursing education that enhances early recognition and management of clinical deterioration. Background - Early recognition of clinical deterioration is a critical skill for undergraduate nursing students, essential for ensuring patient safety, while traditional simulation approaches face challenges with scalability and learner engagement. Virtual reality simulation offers potential solutions but lacks a unified theoretical framework for systematic integration into nursing curricula. Methods - A narrative synthesis of heterogeneous studies on virtual reality simulations in nursing education, conducted between 2015 and 2025, was guided by PRISMA guidelines. A systematic search yielded 20 articles for inclusion. The review mapped relevant learning theories, psychological perspectives, and simulation frameworks, aligning them with outcomes associated with early recognition of clinical deterioration. Results - The synthesis highlighted that virtual reality simulation is effective when anchored with constructivist and experiential learning paradigms. Kolb's Experiential Learning Cycle, Cognitive Load Theory, Bandura's Self-

Efficacy Theory, and Keller's ARCS Motivational Model provided key theoretical scaffolding. Applied frameworks, including Tanner's Clinical Judgement Model, NLN Jeffries Simulation Theory, INACSL Standards of Best Practice, TeamSTEPPS, and PEARLS debriefing, informed simulation design, fidelity, facilitation, and evaluation. These elements converge into a conceptual framework emphasising immersion, cognitive optimisation, motivation, clinical reasoning, and reflective practice. Conclusion - The proposed framework provides a theoretically grounded model for designing and implementing virtual reality simulation in nursing education. It offers practical guidance for educators, establishes testable propositions for future research, and contributes to preparing graduates to recognise and respond to clinical deterioration safely.

PS2.74

Spatial orientation and navigation (Part 2)

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Bees vs spiderwebs: how background contrast affects obstacle detection in stingless bees

For rapidly flying insects, detection of obstacles is essential for navigation and safety. Collisions with obstacles can lead to wing damage and in some cases death. Obstacles vary in salience, however, and the appearance of an obstacle is greatly influenced by intrinsic factors such as the visual system and the insect's flight control, as well as extrinsic factors such as light availability and the background contrast. Therefore detection of low salience obstacles such as spiderwebs, which are thin and translucent structures, is a challenge for most flying insects. Spiderwebs are more visible against darker backgrounds, and almost invisible against lighter backgrounds. In this study, we aimed to identify whether bees flying towards a food source detect spiderwebs against different background contrasts. We trained Mexican stingless bees to fly in a flight tunnel to reach a food source. Trained bees were then tested with orb webs placed before the feeder. Bees encountered webs with two different backgrounds, black and white, which represented high and low contrast scenarios. Subsequently, we tested bees against a cluttered background simulating natural conditions. We filmed bee flights with a high speed camera placed directly above the tunnel. We carried out a frame-by-frame analyses to describe the bee flight kinematics and choreography. We will show how background contrast affects detection of a naturally occurring obstacle by free flying bees.

PS2.84

Learning, memory and cognition

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Visual learning using optogenetics in freely walking *Drosophila*

Vision is essential for a wide range of navigation behaviors in seeing animals, and many species can learn visual features of their environment to guide flexible behaviors. In *Drosophila melanogaster*, numerous studies have demonstrated that flies can associate

visual cues with reward or punishment. For example, learning to avoid or prefer specific objects paired with aversive or appetitive stimuli, or learning to seek refuge from punishment based on the place cues within panoramic visual scenes. However, these experiments often require complex, bespoke setups and specialized knowledge, hindering standardization and replicability of these fascinating behaviors. To address these challenges, we are developing a modular setup that incorporates features of multiple visual learning paradigms with the aim of standardizing these assays. Our system includes a multi-color LED backlight for precisely timed optogenetic and visual stimulation, a new 'G4.1' LED arena for panoramic visual stimulus presentation, and a swappable chamber housing groups of flies within a confined, heat-ring-enclosed space. We will release all the designs, code, and methods as an open science toolkit. We will present our progress implementing visual feature learning assays with this new setup, all using transgenic lines expressing an optogenetic depolarizer in neurons that drive avoidance behaviors: (1) object learning, where discrete visual features are paired with reinforcement in a two quadrant assay ; and (2) visual place learning, where a single safe zone--relief from punishment--is paired with a consistent feature of a visual panorama. Given recent advances in *Drosophila* neurobiology, from driver lines and connectomes to advanced genetic tools, we hope this platform enabling reliable, replicable visual learning experiments will provide a foundation for multi-level investigations into neural circuits linking visual processing with experience-dependent behavior.

PS1.18

Motor systems, sensorimotor integration and behavior (Part 1)

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The universal smile is person-specific

Smiles are the most common and universally recognized facial expression. While we intuitively appreciate people have distinctive ways of smiling, it is unknown whether they are distinct enough to serve as identity markers and are used for that purpose. To test this, we created an ecological dataset of 19,000 smiles from global public figures of a variety of ages and geographic locations. Using neural networks with action unit intensities as features, we demonstrated that people possess unique smile signatures independent of facial appearance. Classification accuracy reached over eight times above chance level, and 80% for pairwise comparisons, but in the absence of a smiles the performance was near chance. This result was confirmed also with logistic regression. While 17 extracted action units were activated during smiling, the six most informative ones were sufficient to replicate these results. Next, to examine whether people are sensitive to the unique individual smile patterns, we used artificial intelligence to project strangers' smile patterns onto still photographs with neutral facial expressions of either familiar people or strangers. This methodology, dissociating facial appearance and smile patterns, allowed us to examine whether smile patterns function as independent identity cues. The participants indicated for each image whether the smile is real or manipulated. We found higher recognition accuracy for familiar compared to unfamiliar faces, and replicated this effect, showing that people have individual smile patterns that are used based on familiarity, similar to an individual's facial or vocal signature. We conclude that there is reliable individual variation in smile patterns that people recognize, such that smiles are not just a means of expression but are affective identity markers.

PS1.67

Spatial orientation and navigation (Part 1)

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Hippocampal coding during real world navigation in bats flying on a remote island

Animal brains have evolved to support complex behavior in natural environments, yet much of our understanding of neural coding stems from experiments performed in the laboratory - with the assumption that neural properties gleaned from simple laboratory experiments can be extrapolated to understand how the brain works in the real world. We aimed hereto test this fundamental scientific assumption, and performed the first single-unit neural recordings outdoors from the hippocampus of freely flying bats as they navigated on a remote oceanic island. We combined this novel neuroethological paradigm with a key advance in modern system neuroscience, and recorded from >150 neurons simultaneously - to investigate the properties of neural manifolds in addition to those of single neurons. Dorsal CA1 hippocampal neurons showed place coding as the animals navigated in the wild, with single place cells having multi-field and multi-scale spatial coding when navigating large areas outdoors, similar to previous findings in large-scale indoor environments. We tracked the bats from the first day they explored this novel geographical area, and found that the fraction of place cells and their spatial coding properties evolved across days. The neural activity manifold in the hippocampus showed a striking difference between real world navigation and restricted navigation indoors - with the manifold dimensionality being much higher outdoors than indoors. This difference could not be explained by the spatial scale of the environment, as it was much higher outdoors than both small and very-large-scale indoor environments. Thus, we provide the first insights on spatial coding in the mammalian hippocampus during real world navigation outdoors, and highlight the need to investigate neural activity in animals performing complex behavior in natural environments to truly understand the structure of neural coding in the brain.

PS3.69

Olfaction, taste and chemical sensing

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Contact-mediated repellency defines Picaridin action in *Aedes aegypti*

Animal behavior unfolds continuously in time, yet quantitative analyses often require segmenting it into discrete, interpretable states. Although manual annotation can achieve this, it remains slow, subjective, and difficult to scale. Most automated pipelines use tracked body parts to infer actions, but are limited by tracking quality, and discard much of the visual information contained in raw videos. Here we present FERAL (Feature Extraction for Recognition of Animal Locomotion), a supervised video-understanding toolkit that bridges this gap by mapping raw video directly to frame-level behavioral labels, bypassing the need for pose estimation. Across benchmarks, FERAL outperforms state-of-the-art pose- and video-based baselines: on a benchmarking dataset of mouse social interaction, it surpasses Google's Videoprism using just a quarter of the training data. FERAL generalizes across species, recording conditions, and levels of behavioral organization: from single-animal locomotion to complex social interactions and emergent collective

dynamics. Released as a user-friendly, open-source package, FERAL overcomes the challenges of traditional approaches, integrates easily with existing analysis pipelines, and can be deployed locally or on cloud servers with a few clicks. By mapping raw video directly to annotated behavior, FERAL lowers the barrier to scalable, cross-species behavioral quantification and broadens the range of behavioral analyses possible in both the lab and the wild.

PS3.95

Methods

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FERAL: a video-understanding system for direct video-to-behavior mapping

Animal behavior unfolds continuously in time, yet quantitative analyses often require segmenting it into discrete, interpretable states. Although manual annotation can achieve this, it remains slow, subjective, and difficult to scale. Most automated pipelines use tracked body parts to infer actions, but are limited by tracking quality, and discard much of the visual information contained in raw videos. Here we present FERAL (Feature Extraction for Recognition of Animal Locomotion), a supervised video-understanding toolkit that bridges this gap by mapping raw video directly to frame-level behavioral labels, bypassing the need for pose estimation. Across benchmarks, FERAL outperforms state-of-the-art pose- and video-based baselines: on a benchmarking dataset of mouse social interaction, it surpasses Google's Videoprism using just a quarter of the training data. FERAL generalizes across species, recording conditions, and levels of behavioral organization: from single-animal locomotion to complex social interactions and emergent collective dynamics. Released as a user-friendly, open-source package, FERAL overcomes the challenges of traditional approaches, integrates easily with existing analysis pipelines, and can be deployed locally or on cloud servers with a few clicks. By mapping raw video directly to annotated behavior, FERAL lowers the barrier to scalable, cross-species behavioral quantification and broadens the range of behavioral analyses possible in both the lab and the wild.

PS2.89

Learning, memory and cognition

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Balancing opposing memories: circuit mechanisms of flexibility in *Drosophila*

Upon experiencing an event, animals store relevant information as memories to guide future behavior. In dynamic environments, new experiences often conflict with previously acquired information, requiring animals to update their internal representations. When faced with such conflict, the brain must choose between forming a new memory with opposing valence or updating the original trace; however, the circuit mechanisms mediating this choice remain elusive.

Using a counterconditioning paradigm in *Drosophila melanogaster* where cue valence is reversed, we demonstrate that conflicting information leads to the formation of a parallel memory that competes with the initial trace to dictate behavioral output. We show that internal states, such as hunger and contextual cues, gate the expression of these opposing memories. Surprisingly, through in vivo calcium imaging, we found that parallel memories

with opposite valences are encoded within the same distinct neural population in the Mushroom Bodies rather than by separate circuits.

Integrating these functional data with connectome analysis, we identified specific circuit motifs capable of maintaining flexible representations of cues with opposing valence. Our findings suggest a potential mechanism for how the insect nervous system encodes dynamic environmental changes and shifting expectations. This provides insight into the evolutionary adaptations for behavioral flexibility and the potential mechanisms disrupted in pathological conditions.

PS3.21

Motor systems, sensorimotor integration and behavior (Part 3)

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Functional analysis of the locomotor circuits in *Drosophila melanogaster*

Coordinated movement requires integration of top-down behavioral goals and fast reflex loops. For terrestrial locomotion in 6-legged insects, the ventral nerve cord (VNC), specifically the thoracic neuromeres, is the site of this integration. To identify circuits controlling coordinated leg movements in the fruit fly, *Drosophila melanogaster*, we created genetic tools targeting VNC local and intersegmental neurons. Screening ~16000 GAL4 combinations, we generated ~1000 stabilized split-GAL4 lines, identifying 508 cell types. We have matched many of these cell types unambiguously to the MANC connectome dataset and for additional lines have identified several possible matches. We estimate (including both unambiguous and ambiguous identification) that our collection targets ~38% of the intrinsic cell types in the leg neuromeres. To study the function of these neurons in locomotion, we selected 797 lines and performed an optogenetic silencing screen using the light-gated chloride channel, GtARC1. We recorded groups of freely walking flies (5 females and 5 males) in a 50 mm diameter, 3.5 mm high chamber designed for high-throughput behavioral screening at 150 fps and 1MP with a custom LED backlight providing optogenetic stimulation and IR for the video recording. Each of the 9000+ videos was processed with our automated Fly Disco Analysis (FDA) pipeline. The pipeline performs body tracking, registration, part tracking of the limbs, behavior classification, and quality control checks. For this project, we have developed an additional module in the FDA to analyze leg-based locomotion phenotypes including the phase relationships of the limbs, as well as spatial and temporal features of limb movement. This allows us to identify the behavioral phenotypes of VNC intrinsic neurons. These data, together with the circuit data from the VNC connectomes, allow us to assign functional roles to specific VNC neurons and circuits involved in limb control and coordination.

PS3.23

Motor systems, sensorimotor integration and behavior (Part 3)

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Ball pythons use cues analogous to visual features to intercept looming infrared targets

Infrared (IR) sensation enables animals to detect objects based on the radiant heat they emit. Among vertebrates, three snake lineages - pythons, boas, and crotalines - independently evolved sensitivity to IR radiation. Classic behavioral studies demonstrate that these snakes can accurately strike stationary targets using thermal cues alone. In our research, we are focusing on the capabilities of the IR system to provide real-time estimates of target position and motion. Using an IR emitter mounted on an XYZ gantry system, we presented looming thermal stimuli of varying sizes and speeds to blindfolded ball pythons. Behavioral responses were quantified using DeepLabCut. Our results show that ball pythons reliably strike approaching IR targets in the absence of visual input in a manner that resembles natural prey interception behaviors. Moreover, preliminary analyses indicate that the timing of strike initiation may depend on the target's apparent size or angular velocity, strategies that resemble the mechanisms by which other animals estimate time to collision using visual cues. We are currently conducting analyses to determine which stimulus parameter best predicts behavioral timing by quantifying the relative position, velocity, and acceleration of both the snake and the target across trials. If IR-sensitive snakes compute looming trajectories using parameters analogous to those in vision, this system could provide a powerful comparative framework for understanding how distinct sensory modalities converge on similar computational solutions to extract behaviorally relevant information. These findings will guide hypotheses about the computations performed by IR-responsive neural circuits, which we will test using electrophysiological recordings. This work represents the first stage of a research program aimed at understanding the neural mechanisms underlying IR function and its integration with vision in the central nervous system.

PS2.21

Motor systems, sensorimotor integration and behavior (Part 2)

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Manipulation of objects by ants

Harvester ants, such as *Messor barbarus*, are central-place foragers: they search for food items that are dragged or carried back to the nest. Food can include seeds, fruit, and other insects, demonstrating the capability of these ants to grasp and transport objects with a wide variety of shapes and sizes. Individual harvester ants manipulate these objects using their forelegs and mandibles, and they are further observed to manipulate nest-mates, larvae and eggs, without causing damage to them. Sensory hairs cover their body, including the legs and antennae, providing tactile information that allows them to manipulate objects even in the darkness of the nest. The ability of ants to manipulate novel objects surpasses current technical solutions in robotics, despite the relatively limited computational power of their small brains. By analysing object manipulation in freely moving ants, we aim to gain insight into stereotypical movement patterns and the transition between behaviour subcategories. We have used a multi-view, high-speed, camera array to record multiple ant-object interactions. This has produced a publicly available dataset which includes video of multiple ant-object interactions, verbal descriptions of the activity in each video, and 2D pose annotation for 26 key points across the ant body, supporting 3D pose tracking. We are adding ethographic behavioural annotations for interactions between the recorded ants and the manipulated object, including the interactions between the ant mandibles, antennae and forelegs. One insight emerging from this work is that the mandibles appear to be mostly utilised in the final stage of object grasping, after a significant process of manipulation by the forelegs to appropriately position the object for grasping.

PS1.21

Motor systems, sensorimotor integration and behavior (Part 1)

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Effects of neurotoxic pesticides on visuomotor control during gap crossing by bumblebees

Bumblebees exhibit remarkable flight performance that relies on the precise visuomotor integration, particularly during foraging and obstacle avoidance in cluttered environments. In agricultural landscapes, foraging bees might get exposed to agrochemicals such as neonicotinoid pesticides, which act on nicotinic acetylcholine receptors, as well as fungicides that may interact with their effects. Despite growing evidence for sublethal effects of pesticides, there is a lack of standardized approaches combining ecologically relevant exposure scenarios with assays capturing naturalistic behaviours under controlled conditions.

To address this, we used a robust yet challenging behavioural assay - a gap-crossing paradigm in a flight tunnel during homing, in which bees need to cross a narrow gap to reach the nest. This task requires complex visual analyses of the position, size, and edges of the gap against a background with similar texture to the chamber walls, and highly controlled flight requiring a sideways turn to avoid wing damage. As such we hypothesize that this task should be sensitive to visuomotor dysfunction. Using this assay, we therefore tested whether sublethal exposure to pesticides, alone or in combination, impairs visuomotor control under varying visual conditions by manipulating background salience to increase task difficulty.
Flight trajectories were automatically tracked using a deep-learning-based pose estimation framework (DeepLabCut), allowing precise quantification of body alignment (head to abdomen axis) and behavioural metrics, including lateral scanning manoeuvres, gaze orientation, body orientation at the gap edge, time spent near the obstacle, and general flight kinematics (e.g., speed and angular velocity).

This assay provides a sensitive, mechanistic approach to quantify subtle effects of neurotoxic pesticides in visuomotor behaviours essential for navigation and foraging and could be extended to other social flying insects such as honeybees and wasps.

PS3.74

Olfaction, taste and chemical sensing

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Ant aggregation pheromones: from social behavior to neural coding
Social insect colonies are among the most cohesive groups in the animal kingdom, yet the signals and sensory mechanisms underlying this cohesion remain poorly understood. Here, we show that cuticular hydrocarbons (CHCs), low-volatility compounds present on the insect body surface, play a central role in social cohesion in the clonal raider ant, *Ooceraea biroi*. The ants deposit CHCs onto the nest substrate, and these chemical residues are sufficient to induce aggregation and to promote group cohesion. Nest-associated CHCs elicit aggregation across distinct *O. biroi* genotypes, indicating that they function as a species-wide signal. In contrast, hydrocarbon mixtures from other ant species or synthetic hydrocarbons absent from the *O. biroi* cuticle do not elicit aggregation. We further show that behavioral responses to nest-associated CHCs vary with age and physiological state and correlate with the ants' propensity to leave the nest. This suggests that this variation contributes to age-based division of labor and other behavioral dynamics in the colony. We identify a specific component of the CHC profile, 11,15-dimethylheptacosane, that promotes aggregation: young workers are attracted to this highly abundant CHC present both on the cuticle and the nest substrate. Together, these findings identify a pheromone-based mechanism underlying group formation, social cohesion, and division of labor in the clonal raider ant. Finally, we describe our efforts to establish a contact-based calcium-imaging set-up to study how perception of this pheromone and related behaviors are encoded in olfactory sensory neurons, using an established transgenic line. Overall, *O. biroi*, with its genetic amenability and evidence of an aggregation pheromone, presents an exciting system to gain a mechanistic understanding of how sensory perception can sustain group formation and social behaviors in ants.

PS2.39

Vision and photoreception (Part 1)

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Oil droplet spectra in the migratory songbird retina: significance for magnetoreception
Night-migratory songbirds can use the Earth's magnetic field to orient during long-distance seasonal flights. It is assumed that their magnetosensitivity is based on a light-dependent, radical-based mechanism that takes place in the retina. Cryptochrome 4a (Cry4a), the most promising candidate for the primary magnetosensory molecule, has been found to be expressed in the outer segments of double cone photoreceptors. For activation it requires light with wavelengths <500 nm, which can potentially be blocked by oil droplets (ODs) - the filtering structures within avian cones. We investigated the spectral properties of ODs within the retina of songbirds and estimated the spectral sensitivity of the cones. For this purpose, we measured the absorbance spectra of ODs using hyperspectral imaging on retinal flat-mounts from Eurasian blackcaps (*Sylvia atricapilla*) in different seasonal states. We show that the blackcap retina has similar ODs as other birds, located in four single cones types (PR1-4) and in the principal member of the double cone (PR5). The ODs of PR5 allow wavelengths >500 nm to pass, but shield Cry4a inside the outer segment from receiving blue photons, likely preventing radical pair formation and magnetoreception. During the migratory season, their cut-off wavelength shifts even further towards longer wavelengths. It is known from behavioural studies that birds can successfully use a magnetic compass under short-wavelength illumination <500 nm. Since the OD of the PR5 would completely block such light, this photoreceptor type cannot be considered a primary magneto receptive cell. This makes the double cone accessory member (PR6), which lacks an OD, the most suitable candidate for this role. The study was supported by the ERC

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PS2.87

Learning, memory and cognition

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The spectrum of artificial light at night shapes the spatial geometry of hawkmoth flight

Decision-making is a fundamental aspect of animal behaviour, with critical consequences for the animal's survival. It enables an animal to refine its behaviour through iterative loops of positive and negative experiences, allowing it to learn. One commonly used model to study learning and decision making are insect pollinators, such as bumblebees. For these insects, learning is a crucial aspect of their foraging, allowing them to identify which flowers are most rewarding. Studies exploring this ability often investigate freely flying bumblebees choosing between artificial flowers with different features, allowing for the collection of a large number of choices. While such choices reflect the outcome of a decision, the information contained in the animal's movement while actively acquiring information is currently underexplored. To study decision-making during flower foraging, we focus on the movement patterns of *Bombus terrestris*. Building on previously described stereotyped behaviours during flower approach, we explore whether learning alters these movement patterns. To do this, we use a learning paradigm associating colour with a sugar reward and extract trajectory features reflecting the spatiotemporal dynamics of flight - such as velocity, viewing angle, and proximity to the flower - and analyse how these covary with learning success. Taken together, we study the sequence of stereotyped movement behaviour in bumblebee decision-making, and discuss how it shapes learning in the context of flower foraging.

PS3.9

Motor systems, sensorimotor integration and behavior (Part 3)

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Song-evoked and premotor activity in female zebra finch HVC

In sexually dimorphic species, brain circuitry essential for male-specific behaviors is often present in females, but its function is rarely explored. Female zebra finches do not sing, but female HVC, a song control nucleus in males, receives auditory input and projects to primary motor nucleus RA and the song basal ganglia Area X, as it does in males. Whether female zebra finch HVC neurons respond to conspecific vocalizations is unknown. To explore this, we performed acute extracellular recordings in 13 awake, head-fixed females and chronic recordings in 9 females housed with a male. Acute subjects heard directed courtship songs from their father, partner, a familiar neighbor male, and an unfamiliar male, plus time-reversed controls. Chronic subjects heard live vocalizations from their partner or father, unfamiliar song and call playbacks, and produced their own calls during sessions. We found robust auditory responses in female HVC. A quarter of acutely recorded units responded to forward song relative to baseline. Reversed songs drove far fewer responses. Introductory notes drove responses in more units than motifs, suggesting some sensitivity to early song structure. Subsets of units responded selectively to one song type. The

direction of these responses depended on the female's relationship to the singer. Father Song-selective units were biased toward excitation, while Unfamiliar Song-selective units were biased toward suppression. In chronic recordings, where females could move and interact freely, a majority of units responded to song. Unlike in the acute recordings, more neurons responded during motifs than during introductory notes, though their firing rates did not differ. Unfamiliar songs that initially drove strong responses habituated over repeated presentations to near-baseline levels. Female HVC neurons also fired before the bird's own calls, consistent with motor drive rather than auditory feedback. This places premotor activity in female HVC upstream of recently reported premotor signals in female RA. Altogether, these results indicate that female zebra finch HVC participates in song perception and vocal production, challenging the longstanding view that female HVC is merely vestigial.

PS2.75

Spatial orientation and navigation (Part 2)

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The behavioral mechanisms governing collective motion in swarming locusts

Collective behavior, from fish schools to bird flocks, is one of the most striking phenomena in nature, yet the local interactions that generate such coordinated motion have remained notoriously difficult to study directly. As a result, the field has been dominated for more than two decades by self-propelled particle models, which reproduce striking correlations with nature. However, the extent to which these models reflect the biological computations performed by individuals has remained unresolved. Here, using juvenile desert locusts as a model organism, we combine field observations from the recent locust outbreak in Africa, laboratory experiments, and a 360-degree immersive, interactive virtual reality system. We show that classical self-propelled particle models cannot account for the emergence of coordinated marching in locusts. By excluding alternative hypotheses, including optomotor responses, we find that collective motion is better explained by a minimal cognitive framework based on consensus dynamics: a ring-attractor architecture grounded in known navigational networks, in which neighbors provide inputs to circuits that govern movement direction. Our study shifts collective behavior from a phenomenological description toward a mechanistic understanding rooted in neural computation, establishes a framework for explicitly testing the causal role of navigational circuits in collective behavior, and opens new opportunities to integrate visual ecology, and bring comparative approaches into the search for universal principles of animal collectives.

PS2.67

Spatial orientation and navigation (Part 2)

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Landmarks or panoramas? dissociable spatial memories in honey bees.

Spatial memory is vital for central-place foragers that navigate the same habitat on a daily basis. Computational models suggest that insects use visual snapshots of the panorama associated with goal directions to recall spatial goals. To what extent these snapshots represent retinotopic images or whether salient landmarks outweigh the rest of the panorama and are stored separately remains unclear. With behavioral experiments, we show that foraging honey bees learn spatial goals by using visual cues in two different ways. Our results indicate that two-dimensional visual patterns and three-dimensional landmarks are learned and used differently during navigation. In a spatial-memory task, walking honey bees had to find a rewarded feeder in the presence of visual patterns or local landmarks. Selectively perturbing the visual scene by adding a novel visual cue degraded goal-directed navigation only when visual patterns were present but not when local landmarks were available. Presenting these patterns and landmarks simultaneously and setting them in conflict revealed that honey bees primarily rely on local landmarks for navigation. Making the spatial information of the local landmarks ambiguous did not disturb their orientation and the honey bees relied on the visual patterns for finding the rewarded feeder. Our findings suggest that honey bees learn different types of visual cues differently in a spatial-memory task. Local cues are learned as landmarks, i.e., uncoupled from the distal panorama while distal visual patterns are grouped into a retinotopic panoramic image.

PS1.62

Social behavior and neuromodulation (Part 1)

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Does body size correlate with the dominant sound frequency of field cricket calling songs?

As a mechanism of female choice for larger males, it was suggested that female field crickets should prefer conspecific males that produce calling songs with lower sound frequency. However, several studies failed to show a correlation between male body size and the dominant frequency of their calls. Male field crickets sing by rhythmically rubbing their front wings, which have specific structures for sound production. During each wing-closing cycle, a sound pulse is produced when the hard edge (plectrum) of one wing scratches over a line of cuticle teeth (file) at the ventral side of the other wing. The resulting vibration of both wings are amplified, phase shifted, filtered and emitted as sound waves by membranous wing areas (chord, harp and mirror). Here we analysed how different body size indicators (body mass, length and width) and the wing structures for sound production (file length, teeth distance, as well as the chord, harp and mirror areas) correlate with the acoustic features (dominant sound frequency, pulse duration, pulse period) of the calling song in two different captive populations of *Gryllus bimaculatus* that differ significantly in body size; relatively large crickets form our lab colony and significantly smaller crickets purchased from a commercial breeder. To test for the impact of the differences in their developmental conditions and genetic background, we also raised offspring from the purchased crickets under our lab colony conditions and produced hybrid crickets by crossbreeding. Both, better developmental conditions and crossbreeding with the lab crickets had significant additive effects on body size, wing morphology and dominant sound frequency. In none of the four cricket groups did the dominant sound frequency correlate significantly with any of the other parameters, but significant negative correlations of the dominant frequency with the body size and wing morphology parameters were found when the data of the four groups were pooled.

PS1.65

Spatial orientation and navigation (Part 1)

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Fine-scale navigation: searching ants use multiple guidance mechanisms

Navigation is of crucial importance for the survival of many species. To guarantee the successful completion of a journey, insects engage in very efficient systematic searches to pinpoint their target. The resulting search patterns are highly structured - systematic - while retaining strong levels of adaptive flexibility. It remains unclear how this sophisticated behaviour is generated by the insect central nervous system. I examine the involvement of several candidate guidance mechanisms in the expression of searching behaviour in the desert ant *Cataglyphis nodus*. Restricting the ability of ants to form visual memories around the nest entrance results in nest searches that retained their systematic structure but suffered from reduced precision. Interestingly, asymmetrical restriction led to asymmetrical searches. This indicates that searching ants employ visually guided steering mechanisms, in which the perceived environment is matched to visual memories. Where such memories are lacking, the steering mechanism is impeded. Further experiments in an entirely featureless visual environment reveal that ants can still perform systematic, area-restricted searches in the absence of meaningful visual input. These results suggest the involvement of innate movement routines. A further mechanism, guidance by path integration, has previously been identified in the literature. Agent-based modelling recapitulates the complexities of natural behaviour, confirming that the identified mechanisms can give rise to sophisticated systematic searching behaviour. More broadly, the outcomes allow us to ask questions of fundamental importance about how brains work to produce adaptive behaviour.

PS1.82

Evolution and development

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Shifts in neurotransmission, synaptic plasticity, and stress-response pathways shape the evolution of cheating in cleaning mutualisms

Mutualistic cleaning interactions illustrate the balance between cooperation and conflict in social behaviour. Cleaner wrasses are tempted to cheat by feeding on client mucus rather than ectoparasites, a behaviour that undermines cooperation and varies widely among species. To investigate the molecular evolution of this variation, we combined behavioural assays with brain transcriptomics across nine species of cleaner wrasses (including dedicated cleaners, Labroides, which clean throughout life, and non-dedicated cleaners, such as Thalassoma, which clean mainly as juveniles) and one non-cleaner outgroup. Across lineages, we found recurrent molecular shifts associated with differences in cheating propensity, converging on three functional axes: neurotransmission, synaptic

plasticity, and stress regulation. Phylogenetically, two cleaner lineages emerged, one leading to mostly dedicated cleaners and one to non-dedicated cleaners, with cheater species occurring in both. Early changes in neurotransmission (within GABAergic and serotonergic signalling) appear to have marked the emergence of cheating across cleaner lineages, whereas subsequent branch-specific modifications fine-tuned synaptic architecture and stress responses (e.g. synj2bp, hsd17b3). Cheater species in dedicated and non-dedicated lineages recruited partly distinct gene sets within these shared functional axes, for example, gad1 and htr1b in dedicated, versus grik5 and gabrb2 in non-dedicated cleaners. Signatures of positive selection further pointed to lineage-specific innovations affecting synaptic plasticity and stress pathways, including itpka and jun (synaptic plasticity) and ramp3 (stress response).These results show that the diversification of cheating strategies among cleaner wrasses arose through repeated modifications of neural signalling and stress physiology, revealing how conflict can drive the evolutionary diversification of social behaviour.

PS2.68

Spatial orientation and navigation (Part 2)

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Ants on a slope can recover heading direction from mechanosensory cues

Insects can encode and recover heading directions from multiples external reference such as compass cues, visual scenes or wind. In darkness, they can also update headings through self-motion cues, although such estimates are not anchored to the external world. Here we show that desert ants learn and recover a heading direction from slope-related mechanosensory inputs. Ants were trained to forage along route running sideway across a steep slope. After a day of shuttling between nest and feeder, individuals were captured at the feeder, blindfolded and released on a test slope oriented in the opposite direction. Their initial departure was directed towards the nest direction as indicated by the slope. We conclude that ants can learn the relationship between a slope and body orientation and use this information to recover a goal direction. We discuss how such information might be integrated in the insect heading-representation system.

PS2.50

Auditory systems and acoustic signaling (Part 2)

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Sound localization and binaural auditory processing in juvenile big brown bats

Big brown bats (*Eptesicus fuscus*) pups are born naked, blind, and with closed external ear canals. In most pups, the ear canals open asymmetrically; however, it is unclear whether this developmental asymmetry influences hearing. To address this, we examined the ears of newborn bat pups (n = 82; 42 females) and found a difference of 1 ± 1 (mean ± SD) post-natal days between when the first and second ear opened. After both ears opened, we recorded auditory brainstem response (ABR) waveforms in a subset of pups at 2, 3, 4, 8, and 16 weeks of age plus 1 year of age and compared the monaural and binaural hearing

thresholds. We recorded ABRs using subdermal needle electrodes placed near the base of the auditory bullae while repeatedly presenting pups with broadband acoustic clicks (bandwidth = 84 kHz; duration = 0.1 ms; click rate = 21 Hz; n = 512 repetitions) and averaging the evoked neural response. The average ABR waveform with its peaks and troughs represents the summed activity of neurons from different nuclei within the auditory brainstem pathway. In bat pups <4 weeks old, monaurally-evoked thresholds were lower in the ear that opened first compared to monaural thresholds in the ear that opened second; however, this monaural threshold difference disappeared by 16 weeks of age. Moreover, binaurally-evoked thresholds tended to be lower compared to monaurally-evoked thresholds. We also measured the sound lateralization ability of older pups in a two-alternative forced choice task and found that pups exhibited an aural bias for noise bursts presented to the ear that opened first. Overall, our results suggest that the asymmetry in the timing of the opening of the external ear canals correlates with an initial asymmetry in auditory thresholds that diminishes before adulthood. This initial auditory asymmetry may have implications for localizing sound sources in young bat pups until their central auditory processing circuits mature.

PS2.66

Spatial orientation and navigation (Part 2)

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Home among the gum trees: visual navigation in an arboreal jumping spider

Navigation is essential for all mobile animals to locate food, mates and return home. Our understanding of arthropod navigation is mostly based on social insects that forage from a centrally located home. Since jumping spiders typically do not have a permanent home, their navigation abilities in the wild have remained largely unexplored. Here, we studied an arboreal jumping spider, *Arasia mullion*, that builds an inconspicuous silken retreat on tree bark to which it returns after every foraging trip, making it a unique system to investigate fine scale navigation strategies in salticids. We used a laser pointer to lead spiders from their retreats to specific locations, and found that spiders return home from almost 40 cm. We provide the first experimental evidence that arboreal jumping spiders use both celestial and terrestrial visual compass cues to steer towards home. Spiders could find the location of the retreat even when it was occluded, suggesting they use local visual cues to pinpoint home. In completely unfamiliar locations, spiders relied on path integration but only travelled a fraction of their home vector. We report these navigation strategies and discuss how they may interact to enable successful homing.

PS3.52

Vision and photoreception (Part 2)

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The functions of double cones and oil droplets in the avian retina

Vertebrates share a common ancestor dating back over 500 million years, whose retina contained rods (‘photoreceptor type 0’, PR0) and four spectrally distinct single cone types (‘red’, ‘green’, ‘blue’, ‘UV’: PR1-4). Of these, PR0, 1 and 4 are retained in mammals. By contrast, most non-mammalian tetrapods retained new tetrapod-specific specializations, including ‘double cones’ (PR5,6) and oil droplets. In diurnal birds, PR5,6 dominate the retinal input. Models suggest oil droplets increase spectral sensitivity and PR5,6 feed luminance channels, but these remain functionally untested. Following our previous description of avian retinal output diversity, we now address how different input channels combine to shape these outputs. Using multielectrode array (MEA) recordings of chicken retinal ganglion cells (RGCs), we applied targeted stimuli to test these structures. Comparing stimuli delivered from the photoreceptor (unfiltered) versus the vitreous (filtered) side revealed that oil droplets reduce light sensitivity and act as long-pass filters. Furthermore, stimulation across different log-intensity levels showed that PR5,6 possess a high dynamic range, providing scotopic luminance signals, possibly through shared rod (PR0) circuits. Using multi-spectral white-noise mapping, we inferred the specific cone inputs to diverse RGC types. We propose an avian signal-processing strategy fundamentally distinct from mammals: rather than primarily relying on ancestral red (PR1) cones, birds utilize PR5,6 for luminance, while single cones (PR1-4) and oil droplets handle precise spectral discrimination. Chicken RGCs multiplex these signals, leveraging all seven photoreceptor inputs.

PS3.77

Olfaction, taste and chemical sensing

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Adaptive odor source localization of the male silkmoth, *Bombyx mori*, under a complex environment using a virtual reality system framework

Navigation to a destination is a fundamental common ability of many organisms. To navigate successfully, organisms must acquire environmental information under dynamically changing conditions and convert it into appropriate behavior. Odor has high persistence and diffusibility, and therefore serves as an important cue for navigation in many species. Even insects with small nervous systems can navigate using odor, and this ability has been widely studied. However, most previous studies have investigated environments in which odor flows relatively smoothly from upwind to downwind. Adaptive navigation behavior in environments containing obstacles that block odor dispersion has not yet been fully analyzed. To investigate insect behavior in such environments, where odor diffusion is strongly disturbed, it is necessary to precisely measure the relationship between sensory input and behavioral output. Therefore, in this study, we adopted a virtual reality (VR) framework that enables high-precision recording of sensory stimuli and behavioral responses during navigation. We focused on odor source localization in an adult male silkmoth, *Bombyx mori*. The male silkmoth can detect a female sex pheromone and identify its location. Our VR system for an insect consists of a behavioral measurement platform that records behavior while providing multimodal stimuli (odor, visual, and wind cues), and a computer-based virtual environment that serves as a space for the insects to navigate virtually. We prepared two virtual environments: one without obstacles (Open) and one with obstacles that disturb airflow (Obstacle). The results showed that even in the Obstacle condition, silkmoths reached the odor source with a success rate of over 80%.Behavioral analysis suggested that in obstacles where odor distribution tends to be sparse, the value of a single odor detection increases for the silkmoth; therefore, the silkmoth may actively move by increasing its movement speed when it detects the odor stimulation. In regions where odor and wind directions did not align, such as behind obstacles, silkmoths appeared to reduce reliance on olfactory cues and instead prioritize movement in the wind direction, enabling adaptation to turbulent environments.

PS2.1

Motor systems, sensorimotor integration and behavior (Part 2)

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Acclimation-induced changes in neuropeptide signaling stabilize neuronal circuit activity

Temperature perturbations are a major challenge for the nervous system because neural function relies on highly temperature-sensitive biochemical and molecular processes. Nevertheless, neuronal circuits in invertebrate poikilotherms can be endowed with a remarkable robustness that enables them to remain functional across a broad range of acute temperature changes. Acclimation studies have revealed that temperature robustness shifts and expands to higher temperatures in warm-acclimated animals, suggesting that neural circuits undergo acclimatory-induced plasticity. While the mechanisms underlying this plasticity remain largely unknown, increasing evidence supports the idea that the actions of neuromodulators contribute to neuronal temperature robustness. Here, we test the hypothesis that acclimation is mediated by changes in neuromodulatory signaling that stabilize neural circuit function during long-term temperature changes. To test this hypothesis, we use the central pattern generating circuits (CPGs) in the crustacean stomatogastric nervous system of green crabs, *Carcinus maenas*, which are acclimated to 20∞C (warm-acclimated) and 10∞C (cold-acclimated) for two months. To test neuromodulator signaling we record 1) the activity of modulatory projection neurons known to release neuropeptide modulators important for temperature robustness in stomatogastric CPGs and 2) the postsynaptic currents elicited by neuropeptide release using two-electrode voltage clamp. Our data indicate that modulatory projection neuron activity is higher across temperatures in warm-acclimated animals, suggesting acclimatory-induced plasticity enhances the activity of these neurons and may strengthen neuromodulatory signaling. Our preliminary voltage clamp data indicate differences in postsynaptic currents between acclimation status, suggesting that acclimation also alters the strength of neuromodulatory signaling (i.e., through changes in neuromodulator release or receptor expression).

PS2.32

Vision and photoreception (Part 1)

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Linking temporal contrast in natural scenes to ON/OFF responses in hawkmoth vision

Animal visual systems are expected to match the statistics of their inputs. For example, natural scenes contain an excess of negative over positive spatial contrasts, a bias linked to asymmetries in visual processing (Ratliff et al., 2010, PNAS, 107, 17368-17373). Likewise, regional chromatic specializations in insect eyes (e.g. dorsally UV vs ventrally green-biased photoreceptor organization in *Manduca sexta*) reflect differences between sky and ground

spectral statistics (White et al., 2003, J. Exp. Biol., 206, 3337-3348). Among visual statistics, the temporal contrast structure in natural scenes, and how it is matched to visual processing characteristics in insects remains less well understood. Here, we combine environmental imaging with electrophysiology to address this question. We analyze natural scene image series recorded with a Nikon D850 during controlled translation across open, semi-open and closed habitats, under different solar elevations and orientations relative to the sun. Using the Environmental Light Field framework (Nilsson and Smolka, 2021, J. R. Soc. Interface, 18, 20210184), image sequences are converted to calibrated radiance in biologically relevant units. We quantify natural scene intensity and temporal contrast magnitudes, including their distributions across elevation angle during translation, and compare the prevalence and magnitude of positive and negative temporal contrasts across temporal reference windows. We set these statistics in relation to contrast-step responses recorded from lamina monopolar cells (LMCs) in the hawkmoth *Macroglossum stellatarum*. LMCs are early visual interneurons that respond to temporal changes in brightness and show distinct responses to dark-to-bright ON vs bright-to-dark OFF contrast steps. We discuss how the response characteristics of the early visual system are matched to the temporal contrast structure of natural scenes.

PS3.100

Methods

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An open-hardware workflow for insect neuroethology

An integrative understanding of animal behavior requires a multiscale approach. Neural circuits for sequential decision-making can be studied with open/closed loop behavior, electrophysiology and neuroanatomy. An experiment spanning these measurements would require a sizeable investment in equipment, thus limiting integrative experiments to well-funded labs and a relatively small set of model organisms. We explored whether neuroethology can be done on a budget, by using open-hardware alternatives to existing technologies. Insect flight behavior is especially amenable to this approach, due to the field's long history of studying complex behaviors, availability of model organisms and fast timeframes. We chose the hawkmoth *M. sexta*, and the fruit fly *D. melanogaster*, as our test species, due to their disparity in size and behavioral timescales. The challenge can be broken up into behavior (cameras, force transducers, VR systems), physiology (amplifiers, positioners, data acquisition) and imaging (fluorescence microscopes, microtomes, optical tables). For each, we modified existing open-hardware solutions from different fields, using off-the-shelf components along with 3D printing to make high quality equipment. For behavior, we use novel one-axis and multi-axis (maltese-cross type) force/torque transducers, and low-cost trackball VR systems. For physiology, we demonstrate a parallel-linkage 3-axis micromanipulator for intracellular recording, a stick-slip nanopositioner for extracellular recording, a DIY pipette puller, and a low-cost electrometer. For imaging, we use DIY microscopes (fluorescence and laser-scanning) for characterizing filled neurons. The combined workflow costs ~$1000. We envision 3 applications of this framework. First, by lowering the activation-energy for exploratory science, we hope that well-funded labs can employ an explore-exploit strategy, wherein open-hardware is used for blue-sky exploration, while a switch to "exploit" these results is made using high-end equipment. Second, we hope to bring cutting-edge behavioral research to undergraduate classrooms, by lowering the risk of damaging expensive equipment. Third, we hope to expand the diversity of organisms studied beyond model species, and into countries with limited resources.

PS2.14

Motor systems, sensorimotor integration and behavior (Part 2)

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Optical mapping of foot-substrate interactions and ground reaction forces in adult *Drosophila*

Foot-substrate interactions and resulting ground reaction forces (GRFs) provide insights for neuroethological studies involving biomechanical models of locomotion. Traditional methods lack the spatio-temporal resolution needed to measure substrate interactions and GRFs in small animals like *Drosophila* larvae and adults. Optical interference-based methods like Elastic Resonator Interference Stress Microscopy (ERISM) and Wavelength-Alternating Resonant Pressure microscopy (WARP) are beginning to fill this gap by mapping GRFs with high resolution at cellular1 and organismal levels2. Here we used ERISM to estimate vertical GRFs generated by stationary adult *Drosophila*. We found that single feet exert 0.42±0.21 ?N (x?±S.D.) vertical force on deformable substrates. We then examined the load-bearing sub-structures of the foot, the claws and hair-like projections called setae. Individual claws and setal sets generated approximately 0.07±0.03 ?N and 0.11±0.08 ?N GRFs respectively. Next, we characterised how reducing setae numbers reshapes local patterns of GRFs, using the NarrowB mutant in which setae develop normally but are excised during eclosion3. These mutants showed a significant reduction in the forces exerted by setal sets. However, this did not lead to a redistribution of forces to claws. In line with previous work3, reducing setae impaired vertical climbing on smooth surfaces, and over certain types of horizontal surfaces, but did not strongly impair walking or climbing on textured surfaces where claws could be deployed. Overall, this work provides insight into the biomechanical roles of sub-structures in fly feet and constraints for biomechanical models, and highlights opportunities for use of optical techniques in mapping substrate interactions in neuroethological and biomechanical studies.

1. Busse F, et al. Biomed Opt Express. 2025 Nov 1;16(11):4617. 2. Booth JH, et al. Elife. 2024 July 23;12(RP87746). 3. Tanaka K, et al. microPublication Biology. 2023.

PS3.86

Olfaction, taste and chemical sensing

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From foe to friend: adapting rove beetle chemosensation for symbiosis with ants

Animals frequently interact across species boundaries to detect and respond appropriately to other organisms. In special cases, these interactions reflect symbiotic relationships in which the symbiont’s nervous system has recalibrated to evaluate its partner organism with newfound importance. A powerful model to study this phenomenon is the rove beetle (Staphylinidae). Most species are free-living soil-dwellers equipped with defenses against ants, their primary predators. But many lineages have convergently evolved symbioses with ants and exhibit host-seeking, rather than defensive, behaviors. This dichotomy facilitates comparisons between free-living and ‘myrmecophile’ beetles to ask how the valence of ants has shifted from threatening to necessary for survival. Here, we focus this question on

the chemosensory basis of detecting ant semiochemicals. We show that the myrmecophile *Sceptobius lativentris* robustly follows trails of its host ant *Liometopum occidentale*, while the free-living *Dalotia coriaria* does not associate with trails and avoids these pheromones when presented in a two-choice assay. Strikingly, *Sceptobius* also exhibits aversion in choice assays, suggesting that trail following represents a contextual interpretation of the cues rather than pure attraction. Because intact and functional antennae are required for both behavioral outputs, we perform single-cell RNA sequencing to identify chemoreceptors with antennal expression. We then use CRISPR and RNAi to manipulate candidate receptors and find that TRPA1 is necessary for detecting trail pheromones and eliciting the associated behaviors. Together, we show that via TRPA1, *Sceptobius* has a preserved ancestral avoidance circuit for ant cues in addition to a trail following circuit triggered by trail-specific conditions. Our findings support duplicating and repurposing detection circuits as one method by which myrmecophiles have evolved their adaptive host-seeking responses.

PS2.88

Learning, memory and cognition

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Colour preference and constancy in the giant Asian honey bee *Apis dorsata*

Tropical pollinators forage in environments where floral resources vary in space and time, requiring flexible strategies to optimise foraging efficiency. Despite the ecological complexity of tropical plant-pollinator interactions, pollinator foraging behaviour remains comparatively understudied in the Asian tropics. One such strategy, floral constancy - the temporary restriction to a single flower type - strongly influences foraging success and plant-pollinator interactions. We aimed to: (1) determine colour preference and constancy in wild colonies of the Asian giant honey bee *Apis dorsata*, (2) test whether reward concentration modulates their preference and constancy, (3) evaluate how quickly learned associations override their colour preferences, (4) determine whether bees can use multiple colour associations simultaneously, and (5) assess whether local floral spectral patterns correlate with colour preferences of bees. Bees trained to a neutral UV-grey stimulus showed a strong preference and high constancy for blue over yellow, suggesting a short-wavelength bias. Because bees from wild colonies may have prior foraging experience, this bias cannot be conclusively interpreted as purely innate. Crucially, low-reward conditions elicited strong blue constancy, whereas high-reward conditions weakened it, demonstrating that reward expectation shapes colour choices. When bees learned that yellow was rewarding, they preferred yellow. Bees sequentially trained to both blue and yellow, visited both colours with no overall preference and no effect of last-trained colour, suggesting that recent experience can disrupt constancy. The community's spectral distribution revealed a strong dominance of short-wavelength flowers, which may be consistent with long-term pollinator-mediated selection linked to the blue preference observed in *A. dorsata*.

PS2.90

Learning, memory and cognition

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Quantifying more sleep after learning in flies using DREAMS app
Sleep and memory circuits have been thoroughly studied in *Drosophila melanogaster* to uncover how sleep helps consolidate memory. Research suggests that the pathway connecting these two cognitive processes interacts in the mushroom body. Building on this, we hypothesized the existence of positive feedback between sleep-promoting and memory-associated neurons. Our results show an increase in sleep in flies that underwent associative conditioning for long-term memory acquisition. However, animals that undergo the same protocol without paired stimuli show no significant differences from naive flies. Long-term memory formation demands energy; therefore, an increase in sleep may help conserve energy or facilitate consolidation. The sleep drive needed for this to happen, could be coming from neurons that engage during learning and/or memory processing. To test this hypothesis, we will employ the genetic tools available in *Drosophila melanogaster*, using lines with drivers from specific pathways that are part of these cognitive functions' mechanism. In parallel, to streamline and standardize data analysis for behavioural studies, I have developed an application in R programming language, which integrates tools from existing publications but requires no coding expertise. This program will enable rapid, standardized, and accessible analysis of data from *Drosophila* Activity Monitoring systems and/or Ethoscopes.

PS2.79

Spatial orientation and navigation (Part 2)

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A global screen for magnetically induced neuronal activity in the pigeon brain
How animals detect the Earth's magnetic field remains a mystery in sensory biology. Despite extensive behavioral evidence, the neural circuitry and molecular mechanisms responsible for magnetic sensing remain elusive. Adopting an unbiased approach we employ whole brain activity mapping, tissue clearing, and light sheet microscopy to identify neuronal populations activated by magnetic stimuli in the pigeon (*Columba livia*). We demonstrate robust, light-independent bilateral neuronal activation in the medial vestibular nuclei and the caudal mesopallium. Single-cell RNA sequencing of the semicircular cristae revealed specialized type II hair cells that express the molecular machinery necessary for the detection of magnetic stimuli by electromagnetic induction. Our data supports a model whereby electro-magnetic input from the semicircular canals activates a vestibular-mesopallial circuit within the pigeon brain.

PS3.62

Vision and photoreception (Part 2)

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From night stalkers to day walkers: diurnal foraging in a nocturnal predator, the ogre-faced spider
Animals benefit from possessing sensory systems well-suited to their environmental niche. However, the enhancement of a sensory system to perform well in a specific niche may come at the cost of performance in other niches. For example, nocturnal animals with visual systems adapted for dim light environments may struggle to perform well in bright day light.

A textbook example of this is the ogre-faced spider (*Asianopis subrufa*). Pioneering work by Blest, Land, and colleagues first illustrated the supreme sensitivity of ogre-faced spider eyes, while also documenting a daily cycle in retinal morphology and physiology, where photoreceptors degrade at sunrise and resynthesize at sunset. These circadian sensory alterations match activity patterns of predatory behavior, where *A. subrufa* transform into motionless stick-mimics during daylight hours, only becoming active hunters following sunset. Unsurprisingly, the ogre-faced spider has been a posterchild for visually mediated nocturnal behavior. Here, contrary to foundational work, I show present-day A. subrufua are facultatively diurnal in foraging behavior. First, I report the frequency and ecological correlates of diurnal foraging in natural populations. I then show the effects of diet and lighting environment on the propensity for *A. subrufa* to forage during the day. Next, I compare physiological and behavioral thresholds of predatory behavior between diurnally and nocturnally active spiders. Taken together, I present a foundational understanding of the causes and consequences of this shift away from strict nocturnality to facultatively diurnal behavior and sensory ecology.

PS3.98

Methods

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A method to collect and analyze 3D voluntary flight trajectories

Flight performance requires complex sensorimotor coordination to respond to changing environmental conditions. Voluntary flight is composed of distinct stages that allow animals to perform a stable takeoff and transition into flight. Though the steps are stereotyped, flexible control of rotation about orientational axes is required for adaptive flight control. We have begun targeted disruption of ascending and descending neurons that contribute to flight coordination using molecular genetic reagents in highly restricted driver lines in *Drosophila melanogaster*. To determine the effects of these disruptions, we have created tools to quantify flight performance. FlyFall is an inexpensive and accessible assay, used by high school and undergraduate students, to characterize the contribution of specific neuron types to flight performance. In this assay, glass vials containing single flies are dropped through a guide tube to a recording stage. Upon the vial landing on the stage, flies 'fall' to the vial floor, and their subsequent voluntary takeoffs are recorded at 600 fps using two synchronized high-speed machine vision cameras. These recordings allow characterization of flight quantity and quality over a specified timeframe. Using an ethogram, students blind to genotype, manually quantify flight quality across four stages from takeoff to final stop. Using recordings from both cameras, we segment wing joints, head, and abdomen using SLEAP. Then, using SLEAP-Anipose, we generate 3D wire frames of flies to quantify by frame, rotational acceleration about the three orientational axes. These two approaches allow us to classify the type and severity of behavioral abnormalities and related variation in flight stability as a function of circuit disruption. To demonstrate utility, we characterize a previously unstudied descending neuron, DNxn155, which projects from brain flight control neuropil to the wing motor control neuropil in the ventral nerve cord.

PS1.25

Motor systems, sensorimotor integration and behavior (Part 1)
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Intrinsic resonance shapes acute but not acclimated neuronal temperature responses
Environmental temperature strongly influences neuronal function in poikilotherms because it alters the biophysical properties of neuronal membranes and the processes underlying neuronal signaling. Nevertheless, some species maintain stable neural circuit activity across wide temperature ranges and can acclimate to new thermal habitats. The mechanisms that support neuronal function during acute temperature perturbations and those that enable longer-term acclimation remain poorly understood. We hypothesize that changes in neuronal excitability are central to temperature robustness and help preserve circuit output during both acute temperature shifts and long-term acclimation. To test this hypothesis, we study the pyloric central pattern generator in the stomatogastric nervous system of green crabs, an invasive species that tolerates large temperature fluctuations in its habitat. In this circuit, neuronal excitability and rhythmic output are determined by the balance of ionic conductances in pacemaker neurons, with the hyperpolarization-activated inward current (Ih) playing a key role in pacemaker excitability and rhythm frequency and structure. Ih also determines membrane resonance, a property that governs how neurons respond to inputs at different frequencies and may shape how circuit activity responds to varying temperatures. We measured membrane resonance in pyloric pacemaker neurons using sinusoidal current injections to determine how neuronal frequency responses change during acute temperature challenges and long-term temperature acclimation (10∞C & 20∞C). Resonance frequency increased with temperature in intact circuits and with synaptic feedback blocked, as well as with suppressed action potentials, indicating that intrinsic membrane properties dominate this response. In contrast, resonance properties did not differ between acclimation groups, suggesting that long-term acclimation does not involve major changes in these intrinsic frequency-determining mechanisms.

PS2.36
Vision and photoreception (Part 1)
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The hawkmoth lamina: shared principles and interspecific variation in parallel processing
Many animals strongly rely on vision, as it provides high-dimensional information about the natural world. To extract relevant features, neural systems filter and categorize the visual input early on to reduce its complexity. In insects, the first visual processing stage of the brain, the lamina neuropil, plays an important role in parallel processing. Its main relay neurons, lamina monopolar cells (LMCs), receive direct photoreceptor input, and shape the contrast, luminance, spatial and temporal tuning of the insect visual system in a cell-type specific manner.

We recently described how contrast, luminance and spatial coding reconcile in the lamina of the diurnal hummingbird hawkmoth *Macroglossum stellatarum* (Bigge et al. 2026 Curr Biol. 36:773-783). To this aim, we provide a novel characterization of hawkmoth LMCs, using serial block-face scanning electron microscopy to reconstruct the anatomical fine structure and connectivity of LMCs in a focal lamina cartridge. We further characterised the functional properties of the main relay neurons (L1 and L2) to the motion pathway in terms

of contrast and spatial processing. Crucially, their two distinct spatial processing functions, lateral inhibition and spatial summation are explained by the density and distribution of their synapses in different lamina layers.

Here, we compare the lamina anatomy of *M. stellatarum* with a close nocturnal relative, *Deilephila elpenor*. We identified their LMC types in the new characterization framework we proposed, and discuss morphological similarities and differences, relating to light adaptations and spatial processing. Furthermore, we discuss striking differences in connectivity between homologous neurons of these closely related species and set these into context with the variability that has been described in lamina morphology and connectivity across Lepidoptera, and other insect groups.

PS2.7

Motor systems, sensorimotor integration and behavior (Part 2)

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Multiple mechanosensors coordinate *Drosophila* response to wind

Fly flight requires rapid sensory processing of multiple stimuli, primarily from the eyes, antennae, and mechanosensory halteres, to stabilize and navigate in complex environments. Recent work has shown that visual input to haltere steering muscles changes their activity and alters the haltere's motion. This in turn modified the haltere sensory input to wing and neck motoneurons, resulting in movements that can change the flight path. Here, we explore the functional relationship between the antennae, haltere, and wings by stimulating the antennae with different directions and speeds of wind while imaging the haltere and wings with high-speed cameras. In quiescent flies, wind stimulation causes active haltere movements, and this reaction is greatly reduced and delayed when the wind-sensing joint in the antennae is stabilized. In tethered flying flies, wind stimulation changes the upper and lower limits of the haltere's arc, and these changes increase with increasing wind speed. This response is modulated by the direction of the oncoming wind. When the antennae are glued, haltere movement responses to wind are much smaller, demonstrating that antennae provide most, but not all, of the sensory input necessary for a wind-related haltere movement. This is the first evidence that antennal input changes haltere trajectory during flight. Our data show that antennae are required for directional steering of the wing and haltere, and that the haltere is required for wingbeat amplitude modulations in response to wind stimuli.

PS1.11

Motor systems, sensorimotor integration and behavior (Part 1)

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When danger strikes: midbrain circuits for defensive behavior execution

Instinctive behaviors - such as escape from threat, or hunting of prey - are critical for an animal's survival and are highly conserved across the animal kingdom. In vertebrates, the midbrain plays a central role in translating sensory information into an appropriate motor

response, with several interconnected structures controlling the initiation and execution of instinctive behaviors. The midbrain cuneiform nucleus (CnF) has classically been associated with the control of high-speed locomotion and its connectivity structure places it in a unique position to control locomotion across different instinctive behaviors. However, whether the CnF contributes to locomotion as a general motor output, or selectively to locomotion in particular behavioral contexts, remains unclear. Using neural activity recordings in freely moving mice, we compared the activity of CnF neurons across different contexts of locomotion- including exploration, hunting and escape -and found that CnF neurons are preferentially recruited during defensive locomotion, in comparison to locomotor bouts during exploration or hunting. Supporting this, optogenetic stimulation of glutamatergic CnF neurons reliably elicited shelter-directed escapes. Furthermore, CnF activity reflected the perceived threat independent of the behavioral outcome, suggesting a role in threat representation. Together, our findings establish the CnF as an important brain region in the midbrain defense circuit, suggesting a role as sensory-motor integrator to gate defensive behavior output.

PS3.83

Olfaction, taste and chemical sensing

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Tracking the plume: video analysis of odor preference in a human-preferring mosquito

Mosquitoes use volatile chemical cues to locate and choose hosts. The human-biting species *Aedes aegypti* has evolved a strong preference for human odor over the odor of non-human animals. Behavioral preference for odors has traditionally been assessed with an olfactometer, where mosquitoes fly to and enter one or more ports containing odor stimuli. Preference is quantified via the number of animals that entered and remained in each port. Olfactometer results often indicate that, even after disrupting co-receptors that mediate odor detection and response, *A. aegypti* human preference remains robust. However, these end-point assays fail to account for the complexity of mosquito host-seeking, which involves fine-scale behavioral sequencing. Specific odor cues mediate multiple stages of attraction, including surging upwind, casting cross-wind, and landing. Therefore, odor preference in an olfactometer may also present via changes in flight path and orientation, not just entering a stimulus port. To better understand how wild-type and genetically manipulated mosquitoes sample and discriminate among odor plumes in an olfactometer, we developed the OlfactoTracker, a system of infrared cameras to record high-speed videos of mosquito flight during a two-choice olfactometer assay. Four cameras record video in a one meter-long plexiglass box, where females are exposed to alternative odor plumes for six minutes. We are using the Braid software for low-latency 3D tracking and analysis of parameters, including time spent in the odor plume, latency to enter the odor port, and integrity of the expected host-seeking behavioral sequence. Based on previous work and preliminary observations, we expect females to surge upwind upon plume entry and cast laterally when exiting. Additionally, we expect to find some minute behavioral defects in mosquitoes with mutated Orco co-receptors, even if port entry is unaffected. Conversely, mosquitoes with mutated Ir8a co-receptors may retain surge and cast behavior, despite being less likely to enter the stimulus port. Our system will greatly improve behavioral understanding of host preference in an aggressive disease vector.

PS2.29

Vision and photoreception (Part 1)

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The role of shiny plant signals and polarization in foraging swallowtail butterflies

Most flowers appear matte, while leaves appear shiny. This difference may aid visually-guided pollinators in foraging. Further, shiny leaves are often more strongly polarized (i.e., have greater 'polarization degree' (PD)). Polarization is a property of light detectable by many invertebrate species, and research into polarization vision has mainly focused on extended-field cues (e.g., sky polarization is crucial in insect navigation). However, the role of polarization in physical objects, such as for plant-based signaling, is not well understood. In particular, PD is theorized to provide a more consistent signal in a complex natural scenery than 'polarization angle', another quantity of polarized light, though this has not been tested behaviorally. We hypothesized that shininess and PD from plant surfaces aid insect pollinators in foraging. We tested this hypothesis in the Asian swallowtail butterfly, *Papilio xuthus*, which can discriminate polarization angles and may perceive polarized light as brightness. In this study, we first examined the innate preference of *P. xuthus* for shiny vs. matte surface textures. We found that preference depended on target color and background contrast. Under some conditions, butterflies could learn to choose the unpreferred target. These results suggest that shininess plays a role during foraging but may be integrated with other visual cues such as color and contrast. Next, we examined butterfly innate preference for lights of different PD. Butterflies showed clear preferences that were dependent on the polarization angle. Specifically, when shown vertically-polarized light, butterflies chose the strongly polarized light (greater PD), but when shown horizontally-polarized light, butterflies chose the weakly polarized light (less PD). Congruent with previous work, this suggests that butterflies perceive PD as brightness. Overall, our study suggests that shiny and polarized surfaces can serve as plant signals for pollinating insects.

PS3.25

Social behavior and neuromodulation (Part 2)

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Exploring the neuronal populations driving mating in malaria mosquitoes through single-nuclei RNA sequencing.

In 2021, globally there were 247 million cases of mosquito-borne malaria, resulting in 593,000 deaths. Despite its global health priority, the neuroscience of mosquito mating behaviour is not well understood. The malaria mosquitoes mating behaviour is sexually dimorphic, occurring at dusk in male-dominated swarms, where males locate females acoustically. While bulk RNA sequencing has identified sex-specific differences in neurotransmitter receptor expression within the mosquito ear during swarming, the underlying central neural circuits driving this dimorphism remain elusive. Here, we utilize single-nuclei RNA sequencing (snRNA-seq) and spatial transcriptomics, leveraging time-specific behavioural states to identify the neuronal populations governing mating. By optimizing the Parse Biosciences platform for *Anopheles* neural tissue, we are generating the first comprehensive transcriptomic cellular inventory of the malaria mosquito brain. Furthermore, by utilizing activity-regulated genes as a readout of sustained neuronal

activity, we are exploring the populations activated during mating. This project's holistic and innovative approaches will significantly enhance our understanding of the neural mechanisms underlying mating behaviour, potentially pinpointing numerous precise and nuanced insecticide targets. Moreover, it will contribute to understanding the fundamental neuroscience question of how sensory information is integrated within the brain to influence behavioural outcomes

PS1.16

Motor systems, sensorimotor integration and behavior (Part 1)

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Neurogenetics of behavioral resilience and susceptibility in fish in changing environments

Animals rear offspring in dynamic landscapes where larvae must navigate complex sensorimotor challenges. This transition is critical for anadromous three-spined stickleback; larvae move from protected nests and paternal care to fluctuating habitats as they develop toward their migration from freshwater nurseries to marine environments. By leveraging divergent stickleback populations adapted to distinct ecological niches, we can investigate conserved neurogenetic mechanisms of resilience supporting larval survival during this "nest-to-dispersal" window. This provides a powerful model for predicting how larval behaviors may adapt or fail in rapidly changing environments.

Using high-throughput tracking (SLEAP), we established a quantitative behavioral timeline from hatching through the juvenile stage, discovering that marine stickleback larvae coordinate their swimming to school shortly after hatching. We are probing the sensory basis of this uncharacterized larval behavior via light/dark trials to distinguish the relative contributions of visual and mechanosensory integration.

To map the underlying functional neuroanatomy, we developed Hybridization Chain Reaction (HCR) probes to visualize cfos and neuromodulatory activity within putative sensory integration centers. We will compare behavior and baseline neural maps against larvae under acute thermal stress, hypoxia, and acidification to pinpoint windows of neurodevelopmental resilience or susceptibility to these environmental changes. In parallel, we are developing CRISPR/Tol2-mediated GCaMP transgenesis for real-time neuroimaging while utilizing Genome-Wide Association Mapping (GWAS) to link candidate genes with these resilient and susceptible phenotypes. By linking behavior to neural and genetic markers, we aim to better predict how larval fish broadly will respond to the physiological demands of a rapidly changing climate.

PS2.54

Auditory systems and acoustic signaling (Part 2)

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Seasonality of signalling effort and antipredator behaviour in katydids
Producing highly conspicuous signals to attract mates is a double-edged sword; both potential mates and predators are more likely to detect them. The 'terminal investment hypothesis' predicts that animals will take greater risks to obtain additional matings when approaching the end of their life to increase their chances of greater lifetime fitness. We tested whether this hypothesis predicted changes in the signalling behaviour of the katydid species *Neoconocephalus ensiger* over its reproductive season and whether changes could be due to decreasing sensory sensitivity to predator cues with senescence. Males produce loud and continuous calling song to attract females. Gleaning bats can also use the song to locate katydids, and katydids pause or cease singing when they detect bat calls. We measured how much katydids sang and their responses to bat calls in a naturalistic enclosure. When exposed to bat calls every two minutes, they did not sing less overall than during a control period, but they often paused briefly during bat call sequences. Their responses and neural sensitivity to bat calls did not change over time, but they sang more as the season progressed. Therefore, katydids took more risks by advertising more for mates later in life, but their reactionary defences and sensitivity to predator cues did not change during this time.

PS2.69
Spatial orientation and navigation (Part 2)
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Foraging success influences foraging motivation in ants
Solitary foraging ants learn foraging routes, and can recall them throughout their foraging life, especially if routes are rewarding. Despite their navigational prowess, previous studies have reported that unsuccessful foraging trips are also part of their foraging life. Surprisingly little is known about whether and how unsuccessful foraging trips affect the subsequent foraging behavior in ants. Here we trained *Cataglyphis velox* ants in their natural habitat on a route with a rewarding feeder at the end. Foragers that visited the feeder frequently, were tested by subjecting them to an unsuccessful foraging trip with no food provided. The subsequent foraging latency - the time foragers remained inside the nest until starting a new foraging trip - was analyzed. For most of the tested ants, one single unsuccessful foraging trip increased their foraging latency to a 25-time manifold. This shows that foraging motivation in ants is highly dependent on the success of foraging trips.

PS1.92
Evolution and development
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Comparative characterization of markers brain injury in woodpeckers and songbirds

Repetitive cranial impacts and mild traumatic brain injuries (mTBI) in mammals induce neurodegenerative pathologies, notably cell death and the formation of neurofibrillary tangles (NFTs). While adult headbutting mammals, such as musk oxen, exhibit extensive hyperphosphorylated tau accumulations (a marker of tau pathogenicity), our preliminary data in wild-caught woodpeckers reveal a surprising absence of these aggregates. This contrast challenges earlier findings based on museum woodpecker specimens and suggests unique neuroprotective adaptations. Accordingly, we hypothesized that woodpeckers demonstrate few tau aggregates and degenerating neurons in mTBI-vulnerable regions compared to non-drumming birds. To test this, we evaluated brain trauma markers across avian species with varying impact ecologies. We compared drumming woodpeckers (downy woodpecker, red-bellied woodpecker, and Northern flicker) to niche-matched, non-drumming songbirds (white-breasted nuthatch and tufted titmouse), alongside chickens. Unilaterally injured zebra finch puncture model, along with PS19 tauogenic mice, served as positive controls for cell death and NFTs, respectively. Histological assessments utilized PathoGreen, Thioflavin-S, and Gallyas staining to detect degeneration, fibrillar aggregates, and NFTs, respectively. Our findings were complemented by anterior pallium transcriptomics to identify differential gene expression of transcripts and pathways linked to cellular damage and stress responses. Overall, both woodpeckers and songbirds exhibited a negligible presence of NFTs compared to PS19 mice. Furthermore, while we observed sparse cell death in these wild-caught birds, it was minimal compared to the extensive damage seen in the injured zebra finches. By contrasting these findings with those of impact-adapted mammals, our work highlights the profound evolutionary heterogeneity of brain trauma resilience and underscores the possibility of novel physiological mechanisms for trauma mitigation.

PS3.29

Social behavior and neuromodulation (Part 2)

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Call-based neural activity in the song control system of non-singing female zebra finches

Female zebra finches (*Taeniopygia guttata*) are unable to sing due to the vestigial development of forebrain song control areas such as the RA (nucleus robustus archistriatalis), a premotor nucleus of the song control pathway. In male zebra finches, RA is also involved in call-based vocal communication in addition to song control. Here, we monitored the activity of RA neurons during vocal communication in freely behaving females using a miniaturized telemetric recording device combined with telemetric audio recording. Neurons in the RA region showed premotor activity associated with stack and tet calls, two innate short-range social calls produced by both sexes. RA units were active when females called to respond to a male partner's call or to initiate a partner's call. However, spontaneous, regularly firing units, typical of male RA, were very rare in females or, when found, showed no association with vocal output. Despite the small number of adult female RA neurons, these neurons are not functionless, but are involved in call-based communication.

PS3.19

Motor systems, sensorimotor integration and behavior (Part 3)

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Extensive glial plasticity in response to seasonal change in the adult avian brain

One major function of neural tissue is to sense environmental change and coordinate the re-establishment of homeostasis. Songbirds provide an excellent model for investigating environmentally-induced changes in the cytoarchitecture of neural tissues: in the songbird Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelli*) transition into breeding season drives a rapid, stereotyped doubling of neurons in a major control nucleus of singing behavior called HVC. As sparrows exit breeding conditions, HVC rapidly degenerates, losing around 60,000 neurons in just one day. During seasonally-induced degeneration of HVC, proliferation of nearby progenitor cells that contribute neurons to HVC increases. To investigate the function of this proliferative event, we used BrdU pulse-chase labeling in vivo to label and track the fate and survival of cells born due during this 'reactive proliferation'. We find a rapid and marked increase in new glia within HVC at two days after induction of circuit degeneration, which exists transiently and declines by four days. We also identify a novel population of neural progenitor-like cells extending beyond their classic niche in the ventricular zone into the avian telencephalon and expanding in pool size with HVC degeneration. Moving forward, we aim to affirm these proliferating cells in the parenchyma can give rise to multiple fate-specified lineages by isolating and culturing this cell population.

PS3.30

Social behavior and neuromodulation (Part 2)

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A rat model of addiction and withdrawal syndrome and the effect of D2/5-HT3 receptor modulation

Alcohol addiction and related disorders remain a major public health challenge, with withdrawal syndrome representing a critical factor in relapse and treatment failure. This project aims to establish and validate a rat model of alcohol dependence and withdrawal, focusing on behavioral manifestations associated with withdrawal syndrome. Rats are exposed to a controlled alcohol administration paradigm designed to induce physiological and behavioral signs of addiction. Following alcohol exposure, withdrawal-related behaviors are assessed using a battery of tests sensitive to locomotor activity, social behavior, anxiety-like behavior, and relapse-like alcohol-seeking. Subsequently, we investigate the effects of pharmacological modulation of dopaminergic and serotonergic pathways. Specifically, ligands targeting D2 and 5-HT3 receptors are administered to evaluate their potential to reduce withdrawal symptoms and relapse-like behavior. These receptor systems are strongly implicated in reward processing, motivation, and the neuroadaptations underlying addiction. By combining behavioral assessment with targeted

receptor modulation, this study seeks to provide insight into the neurobiological mechanisms of withdrawal and identify potential therapeutic strategies for reducing withdrawal symptoms and relapse risk.

PS1.9

Motor systems, sensorimotor integration and behavior (Part 1)

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The evolution of the neural basis of dexterity in deer mice

Variation in neuron number is a potential mechanism by which the nervous system evolves to support behavioral adaptation. Corticospinal neurons (CSNs) are a classic example: an expansion in the corticospinal system in the primate lineage has been hypothesized to underlie their exceptional dexterity. However, the role of CSN number in behavior has been difficult to assess due to the lack of a tractable model system. We compared two closely related subspecies of deer mice (*Peromyscus maniculatus*): forest mice, which evolved the ability to adeptly climb, presumably to support a semi-arboreal lifestyle, and prairie mice, which are less proficient climbers. We find that forest mice have about two-fold larger corticospinal tracts (CSTs) driven by an increase in CSN number in secondary motor and sensory cortical areas (M2 and S2). Furthermore, forest mice display greater manual dexterity than their prairie counterparts in a reach-to-grasp task, consistent with the idea that an increase in CSN number supports more dexterous behavior. High-throughput neural recordings during this task revealed a difference in the timing of neural activity between forest and prairie mice, specifically in M2: in forest mice, the peak of activity was shifted towards the grasping phase of the behavior. Forest mice also outperform their prairie counterparts on an ecologically relevant climbing task, where they spend more time upright crossing a thin rod, move faster, and right themselves more quickly when they fall, suggesting a general difference in motor dexterity not restricted to hand use. Finally, we use F2 hybrid animals to show that CST size is correlated with climbing dexterity, providing support for the long-standing hypothesis that corticospinal system expansion supports the evolution of dexterity. Together, our work establishes the forest-prairie deer mouse system as a model to investigate the role of neuron number expansion, and CSNs in particular, in dexterous movement.

PS1.26

Social behavior and neuromodulation (Part 1)

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Transcriptomics of neuro-reprogramming: parasites exploit plasticity in developing social wasps

Parasites have been referred to as evolution's neurobiologists due to their incredible ability to manipulate the nervous systems of hosts. A potential manipulation mechanism is to target the adaptive plasticity of hosts to develop an extended phenotype. Here, we characterized the timing and neuromolecular changes induced by the twisted-wing insect *Xenos peckii* (Strepsiptera) to manipulate developing social wasps, *Polistes fuscatus*. To complete their life cycle, these parasites reprogram short-lived female workers into long-lived phenotypes similar to gynes, while hindering ovary development and cooperative behavior. First, we used a developmental-time series of naturally infected and uninfected wasps to uncover if parasites manipulate the intrinsic developmental plasticity that evolved to produce queen or worker. We confirmed that parasite larvae infect most host larval instars. We also found robust stage-specific differences between uninfected workers and gynes, specifically in genes involved in neural development, insulin and dopamine pathways, and immune responses. Second, we experimentally infected fourth instar larvae, the most common attacked instar, while removing the effect of differential nutrition and social interactions towards caste-bias. Compellingly, parasitic manipulation of neural and caste genes remained consistent. Finally, experimentally infecting long-lived gynes that are not targeted in nature showed minimal parasitic exploitation. Therefore, these parasites exploit specific plastic traits associated with neural development and caste-bias that facilitates an ideal, long-lived host. Our findings reveal how stage-specific targeting during development initiates neuro-reprogramming of hosts towards the final extended phenotype that benefits the parasite.

PS1.34

Social behavior and neuromodulation (Part 1)

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Sex-specific hormonal control of breeding aggression in *Gymnotus omarorum*

Agonistic interactions arise from competition among conspecifics for limited resources and are strongly influenced by sex steroids. The gymnotiform fish *Gymnotus omarorum*, native to Uruguay, is a well-established model for studying non-breeding territorial aggression in both males and females. However, aggressive interactions during the breeding season and their endocrine modulation remain poorly characterized. Here we describe breeding-season agonistic encounters in male and female *G. omarorum*. Intrasexual dyads (24 male and 22 female pairs) of wild-caught fish were observed under semi-natural conditions after short-term isolation in their natural habitat. In both sexes, contests escalated into intense aggression and consistently resulted in the establishment of a dominant–subordinate hierarchy. Subordinates emitted electric communication signals (chirps and offs) that indicated their social status, and males and females differed in how these submissive signals were used during contests. To examine hormonal modulation, estrogen synthesis was acutely blocked with fadrozole injections administered 30 minutes before interactions (male dyads: 7 control, 11 treated; female dyads: 10 control, 12 treated). Estrogens influenced the motivation to engage in fights in females and modulated aggressive behavior after hierarchy establishment in males. In addition, androgen receptors were blocked with cyproterone acetate (male dyads: 10 control, 8 treated; female dyads: 6 control, 7 treated). This manipulation selectively affected the emission of offs by male subordinates but had no detectable effect in females, revealing sex-specific androgenic modulation of submissive signaling. Together, these results show that estrogens and androgens rapidly modulate breeding-season aggression in *G. omarorum*, and that this endocrine regulation is sexually dimorphic during the breeding season, contrasting with the sexual monomorphism reported for non-breeding aggression.

PS2.104

Education

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Teaching neuroethology using problem-based learning

Problem-Based Learning (PBL) is an instructional methodology first used in medical education. In PBL, students work in groups on a case study or problem set. As a group they identify any knowledge gaps, then independently research the topics. In a follow-up session students discuss their findings and possible solutions. This is mostly a student-driven process, a tutor is present for guidance. PBL is a suitable method to teach Neuroethology. Examples of neuroethology case studies suitable for PBL are 1) How do bats deal with Doppler effect in their echo's? 2) What happens at a cellular level in learning. 3) Why are neurons involved in escape responses often so big?

PS3.81

Olfaction, taste and chemical sensing

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Investigating the role of microRNAs on behavioural shifts in *Aedes aegypti*

Female *Aedes aegypti* mosquitoes undergo precisely timed behavioural transitions across their reproductive cycle. They actively seek humans for a blood meal, then strongly suppress host-seeking while searching for water to lay eggs, after which their host-seeking drive is regained. Previous studies have shown that blood-feeding triggers gene expression changes in mosquito brains, but the origin of these changes and their impact on behaviour remain unclear. In other animals, including *Drosophila*, microRNAs (miRNAs) regulate behavioural transitions by silencing specific messenger RNA (mRNA) targets. In *A. aegypti*, a few miRNAs have been shown to influence aspects of the reproductive cycle, such as blood digestion and egg production. However, their potential roles in behavioural control, particularly in the brain where sensory information is integrated, has not been systematically characterized. Here, we are testing the hypothesis that miRNAs coordinate behavioural state transitions in *A. aegypti* brains. Currently, I am profiling brain miRNAs and mRNAs across the blood-feeding cycle to identify candidate regulatory interactions. I am also developing a genetic system to assess how neuronal depletion of the miRNA pathway affects host-seeking, water-seeking, and egg-laying behaviours. These behaviours will be measured using assays that measure attraction to human odour and humidity cues, blood-feeding success, egg production, and egg-laying site selection. By integrating transcriptome profiling with genetic perturbations, this work could uncover post-transcriptional regulatory programs that orchestrate behavioural switching in mosquitoes.

PS1.73

Spatial orientation and navigation (Part 1)

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Quantifying polarization-driven flight behaviour in *Culex pipiens*
Mosquito aggregations range from resting assemblies to aerial mating swarms, yet the sensory computations generating these spatial structures remain unresolved. Swarms are particularly difficult systems in which to isolate sensory rules because multiple feedback processes (acoustic interactions, mating behaviour and density regulation) simultaneously shape group structure. Interestingly, a phenomenon known as swarm site fidelity, implies that mosquitoes may use a combination of these cues in order to return to the same site every day to form aggregations. We therefore study *Culex pipiens*, where individuals were recorded under controlled conditions above surfaces producing strongly or weakly linearly polarized reflections (high vs low degree of linear polarization, DoLP), altering optical information without changing geometry. By tracking their flight dynamics, we quantified how visual input modifies flight kinematics, spatial occupancy and persistence over time. Mosquitoes preferentially occupied high-DoLP surfaces (probability = 0.58, 95% CI 0.52--0.63; GEE: $z = 2.73$, $p = 0.006$; mean PI across recordings = 0.10 ± 0.19 SD). Polarization structure therefore reshaped movement decisions, producing predictable changes in occupancy dynamics rather than overall activity. This work establishes a computational baseline for mosquito collective behaviour: before testing complex mating swarms, we first identify the sensory algorithm in a simpler behavioural context. The framework provides a transferable model that can later be evaluated in swarm-forming species to determine whether mating swarms represents a specialized behaviour across species

PS2.83

Learning, memory and cognition

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Dissecting aminergic contributions to visual discrimination learning in honey bees using a virtual reality paradigm.
The honey bee is a powerful model for studying visual cognition in insects. Over the past decades, numerous studies have explored how bees detect, recognize, and discriminate visual objects. However, dissecting the contribution of specific neuromodulatory pathways during visual learning has proven challenging because most experiments rely on free-flying bees, in which invasive neurophysiological approaches are not feasible. To overcome this limitation, we used a closed-loop virtual reality (VR) system in which tethered honey bees walk on a spherical treadmill while actively navigating a controlled 3D visual environment. Neuromodulatory pathways were pharmacologically manipulated through microinjections delivered via the median ocellus. This approach enables the study of visually guided behavior while maintaining experimental access to the nervous system. Using this approach, we investigated the role of dopaminergic and serotonergic signaling in bees trained to discriminate landmarks of different colors. Pharmacological activation of

dopamine receptors accelerated the emergence of stable discrimination between rewarded and non-rewarded visual stimuli, whereas dopamine receptor blockade strongly impaired both acquisition and short-term memory expression. In contrast, serotonergic manipulations produced subtler, dose-dependent effects: increasing serotonergic signaling did not support stable discrimination learning, whereas receptor blockade resulted in a non-monotonic pattern in which learning was impaired at low doses but partially restored at higher doses. By combining immersive VR with targeted neuropharmacology, this study provides new insights into how distinct aminergic systems shape visual learning in actively behaving insects. More broadly, our results highlight the potential of VR paradigms to link neuromodulatory function, cognition, and behavior under conditions that preserve self-generated movement and sensory feedback.

PS1.56

Social behavior and neuromodulation (Part 1)

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Exploring the role of active antennal movements for sensing courtship songs in flies

Animals use sound to find food, locate mates, and avoid predators. In the fly *Drosophila melanogaster*, sound is sensed and generated during courtship where males 'sing' by vibrating their wings to produce continuous sine waves (sine song) that alternate with intermittent pulses (pulse song). Females respond to the songs with gradual immobility leading to copulation, and males exhibit aggressive behaviors towards other males. Flies detect near-field sounds with mechanosensory Johnston's Organs Neurons (JONs) housed in their antennae. Because flies actively position their antennae for enhanced mechanosensation, we hypothesized that hearing in flies involves active sensing mechanisms which serve to tune JON sensory gain for detecting ethologically relevant auditory stimuli. To test this, we played back sine and pulse song components of courtship songs to tethered and head-fixed Canton-S flies and filmed their antennal movements. Flies exhibited ventro-posterior antennal deflections in response to pulse songs, but produced no movements in response to sine songs and white noise control stimuli. The mean antennal deflection is significantly higher in both virgin and mated males, and mated females, when compared to virgin females. To test whether sound source direction affects antennal movements, we played sound from 7 directions around the fly (-145∞, -90∞, -34∞, 0∞, 34∞, 90∞, 145∞), and observed that the ventro-posterior active movements are consistent for all sound source directions and remain bilaterally symmetric. We hypothesize that the direction-independent active movements in response to pulse songs serve to enhance the directional range of sound localization during courtship, and will test this in future experiments using high-speed videography and in-vivo physiology.

PS3.55

Vision and photoreception (Part 2)

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Polarisation vision in octopus – analogy with colour vision and ideal observer models

Octopuses have polarisation vision but cannot see colour. In contrast, fishes have colour vision but cannot see polarisation. Since fishes and octopuses share the same habitat these two visual systems solve similar problems. Moreover, a colour-blind octopus can match colours of surrounding objects. Hence, it was long hypothesised that polarisation

vision is functionally similar to colours vision. While colour vision is achieved by comparing signals of photoreceptors tuned to different parts of the spectrum, polarisation vision is achieved by comparing signals of photoreceptors tuned to different orientation of polarisation. Therefore, from a theoretical point of view, polarisation and colour vision are similar, with polarisation sensitivity being analogous to chromatic sensitivity. We consider the ideal observer models of polarisation vision that are analogous to the Helmholtz model of colour vision, which does not separate chromaticity and luminance processing, and to the RNL model of colour vision, which assumes separate processing of chromaticity and luminance. While the Helmholtz model fails to describe colour thresholds, the RNL model describes colour thresholds in many animals. To find if octopus processes polarisation separately from luminance, we measured polarisation and luminance thresholds. Reaction to the stimuli was recorded using a ?xation re?ex. The shape of the dependence of luminance and polarisation sensitivity on spatial frequency were identical. This is at odds with the predictions of the RNL model but agrees with the predictions of the Helmholtz model. We also found that the Helmholtz model correctly describes both luminance and polarisation thresholds dependence on the angle of polarisation. We conclude that, in octopus, polarisation and luminance processing are mixed. This contrasts with the separate processing of chromaticity and luminance in fish indicating fundamental differences between processing of polarisation and colour.

PS1.94

Mechano- & thermoreception

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Predator-prey dynamics sculpt intricate mechanoreceptor array in a sea snake

The olive-headed sea snake, *Hydrophis major*, is one of the rare predators to almost exclusively target eel-tailed catfishes (Plotosidae), which sport large venomous spines anterior to their dorsal and pectoral fins. These spines are a real threat to the snake and have led to a sensory arms race: Where the prey has developed venomous defensive organs, we have discovered that the predator has developed an intricate mechanoreceptor array.
Mechanoreception is widely recognized as an important sense in navigation and prey recognition in various aquatic lineages, including pinnipeds via their whiskers and crocodilians via their integumentary sensory organs. However, it remains widely understudied inobligate marinesea snakes (Hydrophiinae), where mechanoreceptors have adapted from flat (ancestral, terrestrial) to dome-shaped protrusions on the skin.

We used gel-based 3D profilometry (Gelsight) to rapidly quantify mechanoreceptor distribution over the head. Both light and scanning electron microscopy were used to further investigate mechanoreceptor morphology and ultrastructure on select head scales.

We show that gel-based 3Dprofilometryisanexcellent tool for rapid, non-destructive imaging of surface sensory organs. It has revealed an intricate sensory array of mechanoreceptors along the mouth, which is a clear adaptation for the handling of spiny catfish, as swallowing them tail first cause serious injury to the snake. Microscopy revealed at least two different types of mechanoreceptors: the smooth domes which are likely mainly for tactile

perception, and the newly discovered asymmetrical peaks which likely operate as a class I lever system and serve hydrodynamic sensing purposes. In the predator-prey arms race with catfishes, asymmetrical peaks appear as directional flow sensors as opposed to non-directional smooth domes. As such, they may be able to detect minute water displacements generated by fleeing catfish and pinpoint their location.

PS1.6

Motor systems, sensorimotor integration and behavior (Part 1)

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Behavioral responses to body rotation in flies using micron-scaled linear oscillation

In flying animals, sensory feedback encoding body rotation improves the performance of rapid stabilization reflexes. Dipteran insects such as *Drosophila melanogaster* possess modified hindwings called halteres that act as gyroscopic sensors, providing rapid mechanosensory feedback that adjusts wing kinematics in response to perturbations during flight. However, the operating range and dynamics of these compensatory reflexes for different rotational axes (i.e. yaw, pitch, and roll) remain incompletely explored. One technical challenge is the difficulty of delivering high-speed rotations to tethered flies. We addressed this limitation by implementing a clever strategy developed for studies of head reflexes in blow flies (Nalbach 1994). The system generates controlled virtual angular velocities by linearly oscillating the fly's body in phase with the haltere motion, thereby reproducing the lateral forces experienced by the halteres during rotation in free flight. Linear oscillations of only a few microns produce virtual rotations exceeding 1000 deg/s, making the technique compatible with both electrophysiology and optical imaging. During tethered flight, we measured head and wing kinematics to quantify haltere-based stabilization reflexes at angular velocities up to 2000 deg/s. We identified persistent steering responses following yaw perturbations, suggesting temporal integration in this motor-sensory pathway, consistent with prior models derived from free flight perturbations (Ristroph et al., 2010). Our data were accurately fit by a simple proportional-integral (PI) model, indicating the presence of an integral component specific to yaw but not pitch or roll. Together, our findings confirm that the halteres function as Coriolis sensors during flight and provide a robust method for investigating the neural basis of compensatory reflexes in flies.

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PS3.76

Olfaction, taste and chemical sensing

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The sensory organization that drives mosquito host detection

The increased prevalence of mosquito-borne diseases is a major public health issue, and an effective strategy to prevent their spread is to avoid mosquito bites. *Aedes aegypti* mosquitoes rely heavily on odor, carbon dioxide, and other chemosensory cues to locate humans, which they detect through chemosensory receptors on their antennae, maxillary

palps, and other sensory tissues. In order to study the chemoreceptors that mosquitoes use to detect humans in vivo, we must first know their location in mosquito sensory tissue, including which neurons house them. *Aedes aegypti* has hundreds of chemosensory receptor genes, and there is no comprehensive map of their expression. To create receptor-to-neuron maps in mosquito sensory tissue, we are using Xenium spatial transcriptomics. We have designed a 300-gene panel to examine receptor expression in mosquito antennae, which we have used to detect transcripts in *Aedes aegypti* antennae. We validated this approach by comparing the transcripts detected with spatial transcriptomics with both bulk and single nucleus RNA sequencing data, and find these methods consistent. We have transformed these data into a 3D-map and illustrated the distribution of receptors in antennal segments and cells along the antenna. Moving forward, we will identify their relationship to landmarks in the tissue. This work establishes a methodology for spatial transcriptomics in mosquitoes and will generate essential receptor-to-neuron maps needed to study chemosensation in *Aedes aegypti* and other mosquitoes. These results will guide future work, to determine whether there changes in receptor expression and distribution occur between different behavioral states, sexes, and species.

PS1.27

Social behavior and neuromodulation (Part 1)

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Age and nutrition shape courtship dynamics in female *Drosophila*

Age, nutrition, and mating experience are important influences on reproductive behavior. In particular, theory predicts that poor physiological condition decreases the stringency of mate choice. This phenomenon is known as mate-choice plasticity and has been observed in multiple mating systems. However, the neurological mechanisms linking physiological condition to mate choice remain poorly understood. Here, we use *Drosophila melanogaster* to begin to elucidate the neural mechanisms underlying mate-choice plasticity, by investigating the effects of female age (0-day-old vs. 10-day-old) and nutritional status (fed vs. malnourished) on female preference for male body size. We measured the time to female orientation, courtship latency and duration, and copulation latency and duration. Preliminary results indicate decreased mate-choice stringency in older and malnourished females. Collectively, these findings support the hypothesis that female reproductive behavior is shaped by the interaction between physiological state and a female's assessment of male quality. While similar patterns of condition-dependent mate choice have been observed in other systems, establishing these effects in *D. melanogaster* provides a powerful model for applying functional genetic, physiological, and neurobiological tools to dissect the underlying mechanisms. Future experiments will examine the role of the Adipokinetic hormone (Akh) pathway in mediating the effects of nutritional status on mate choice. More broadly, future research will leverage the genetic and neurobiological tools available in *D. melanogaster* to identify the signaling and neural pathways underlying mate-choice plasticity.

PS3.87

Electrosensory systems

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Efficient representations of fast dynamic stimuli in populations of ON- and OFF-cells
Neuronal information processing is metabolically expensive. Neurons need to be maintained and neuronal signaling requires ATP, the currency of metabolic energy [1]. For instance, visual information processing requires a significant share of the body's total energy budget, although the corresponding brain structures represent only a small fraction of the body mass. Thus, there is a large evolutionary pressure to minimize the metabolic cost of neuronal processing, while retaining as much information as needed to achieve an accurate representation of the sensory scene and to successfully guide behavior. By splitting the stream of sensory information processing into ON- and OFF pathways, the metabolic demands of encoding sensory signals can be reduced [2]. This architecture is observed across various modalities, including vision, thermoreception, and electroreception. In the electrosensory system, sensory afferents encode densely with extraordinarily high firing rates. However, neurons of the ON- and OFF pathways in the next central processing stage exhibit substantially lower firing rates, which is limiting the information transfer. Therefore, the representation of behaviorally relevant high-frequency information in single cells is attenuated. We evaluated the stimulus encoding performance in the ON- and OFF- pathway by means of model simulations and theoretical considerations. Using a leaky integrate-and-fire (LIF) model, we show that the encoding bandwidth is indeed reduced as firing rate decreases. Combining responses of ON- and OFF cells does not rescue this loss in high-frequency information. However, low-frequency information is better represented in ON-OFF populations. These findings raise the question of how behaviorally relevant high frequencies are encoded in higher order processing stages of the electrosensory system [3,4].

[1] Laughlin & Sterling, MIT press; 2015 [2] Gjiorgeva et al., PLoS Comp Bio; 2019 [3] Henninger et al., J. Neurosci; 2018 [4] Barayeu et al., iScience; 2023

PS2.19

Motor systems, sensorimotor integration and behavior (Part 2)

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Differences in temporal coding in dipteran flight muscle
The evolution of flight was a key driver of insects' widespread success, enabling them to radiate across diverse ecological niches. Of the four independent origins of active flight in animals, insects are unique in that their wings did not evolve from modified forelimbs; instead, insect wings and their associated motor systems represent evolutionary novelties with no clear homologs. Previous work has provided significant insight into the function of these wing motor systems, but primarily in a handful of model species such as the fruit fly (*Drosophila melanogaster*). Broadly understanding the evolutionary trajectory of insect flight motor control will require comparative studies with greater coverage of the insect phylogeny. As a first step toward this goal, we present a comparative investigation of the wing motor systems of fruit flies and mosquitoes. Both belong to the order Diptera yet exhibit distinct patterns of wing movement, making them a strategically chosen pairing for comparison. Using extracellular electrophysiology combined with high-speed videography,

we recorded from the wing muscles of both species during free flight and identified fundamental differences in the temporal patterning of neural input to the wing muscles. We then examined multifunctional control of wing movement by recording from mosquito flight muscles during simulated aerial courtship, contrasting these findings with previous work on the terrestrial courtship of *Drosophila*. Together, these results lay a foundation for future comparative studies aimed at understanding the evolution of the dipteran flight system and insect flight more broadly.

PS1.51

Social behavior and neuromodulation (Part 1)

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No tonotopic organization observed in the turtle hindbrain

The auditory endorganin turtles, the basilar papilla, is tonotopically organized (Crawford and Fettiplace, 1980). However, our previous work on the primary auditory nucleus, the nucleus magnocellularis (NM), and the secondary auditory nucleus, nucleus laminaris (NL), did not reveal obvious tonotopy(Willis & Carr, 2017).We have followed up on this study to test the hypothesis that there is tonotopic organization in the turtle hindbrain that follows the tonotopic organization of the basilar papilla.

We injected dye into the basilar papilla of *Trachemys scripta elegans* to characterize the projections of the VIIIa nerve. Data from these experiments were combined with stereologically guided physiological recordings from the acoustic tubercle, and neurobiotin iontophoresis to confirm recording locations. We included data from Willis & Carr (2017) in our analysis.

The tract tracing from the basilar papilla revealed no clear tonotopy in NM and NA but instead showed label throughout the rostrocaudal extent of NM and NA. Labeled auditory nerve fibers were highly branched and formed dense terminals in both NM and NA. Physiological recordings from the acoustic tubercle revealed sharply tuned, phase-locked responses and moderate levels of spontaneous activity in NM. Iso-intensity frequency response curves showed that most neurons had best frequencies below 300 Hz but some up to 500 Hz, consistent with recordings in Willis and Carr (2017). We normalized rostro-caudal and medio-lateral position of each recording site with respect to landmarks on the acoustic tubercle, and measured depth dorso-ventrally relative to the surface and found no obvious tonotopic organization. Tonotopy may not need to be preserved in projections to the central auditory system in animals with a limited range of frequency responses and precise temporal coding.

PS2.40

Vision and photoreception (Part 1)

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Visual prey categorization in the jumping spider brain
Many animals use visual information to classify objects, including distinguishing palatable prey from aposematic prey that signal unpalatability with high-contrast patterns. Key questions include what visual information predators use and how it is represented in the brain. We addressed these questions in jumping spiders (Araneae: Salticidae), visually guided predators that encounter insect prey varying widely in value and appearance. Salticids have motion-detecting secondary eyes whose visual fields overlap with those of the principal eyes, which provide high spatial resolution and color vision. The principal-eye retinas have small visual fields but move to scan objects. We first presented freely moving *Phidippus audax* with diverse images of palatable and aposematic insects while tracking their behavior. Next, we monitored gaze direction in restrained spiders using a custom-built eyetracker while recording neural activity from lower- and higher-order visual brain areas. Spiders viewed the same prey images along with object-category controls and stimuli with stepwise complexity reductions to test whether responses depend on local features, holistic structure, or object categories. Our results show that behaviorally, spiders were more likely to orient toward and spent more overall time inspecting palatable prey compared to aposematic prey. Neural activity paralleled this pattern. Multi-unit activity increased strongly in response to palatable prey but was suppressed by unpalatable prey relative to the baseline visually evoked response. Among 22 single units recorded across visual brain areas, firing rates were ~3x higher when viewing palatable versus unpalatable prey. Ongoing analyses examine responses to simplified features while correlating retinal inspection patterns with single-unit neural activity. Together, these results reveal neural correlates of prey categorization that may contribute to decision-making in an invertebrate predator.
PS2.95
Learning, memory and cognition
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Neuromodulation of olfactory learning in the whip-spider *Phrynus marginemaculatus*
Amblypygi are an order of arachnids colloquially called whip-spiders because of their modified forelegs, but these appendages are not used for lashing. Instead, they are packed with chemoreceptors and used in active sensing, much like antennae in insects. Also, like insect antennae, these antenniform legs contain chemosensory neurons that terminate in olfactory glomeruli in the whip-spider deutocerebrum and send projections to the mushroom body. These olfactory inputs are segregated from visual inputs in the mushroom body calyx, an organization which has only been described in one other arthropod order: the Hymenoptera (bees, wasps and ants). Bees in particular have been long studied in the context of olfactory learning and the role of neuromodulation in their mushroom bodies in mediating this behavior. Are the same neuromodulators expressed in whip-spider mushroom bodies and are they similarly necessary for olfactory learning? In whip-spider (*Phrynus marginemaculatus*) brains, we explored the expression of a suite of neuromodulators commonly found in insect mushroom bodies and particularly focused on localization of serotonergic, dopaminergic, and GABAergic neurons which were found in the mushroom body calyx. To understand whether serotonin and dopamine are necessary for whip-spider olfactory learning, we trained individuals in an associative task where odors are paired with an accessible shelter. Whip-spiders injected with saline after training learned to associate an odor with the shelter, while this learning was abolished in individuals injected with a serotonin or dopamine receptor antagonist, while their overall rate of locomotion was

the same. This suggests that serotonin and dopamine are necessary for olfactory memory consolidation in whip spiders. Both our neuroanatomical and behavioral results echo motifs found in insect learning and memory circuits.

PS1.39

Social behavior and neuromodulation (Part 1)

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Do locusts retain lateralization individuality throughout gregarization?

The solitary-gregarious phase transition in swarming locust species is a hotbed of behavioural contrasts. During this period, lifestyle strategies- traits like inactivity and crypsis, which advantage living in quiet isolation, must be re-optimized for existence amongst thousands of conspecifics. Despite their scale, these gregarious swarms are far from homogenous, and variability in the individuals which constitute them can shape their movement and orientation. Where does this variation arise- de novo, during the gregarization process, or from pre-existing elements in the solitary population?

We seek to use context-specific lateralization, a metric divorced from behavioural traits directly associated with gregarization, to construct behavioural profiles for individual locusts before, throughout, and after conspecific crowding and to determine how these behaviours shape and are shaped by gregarization. The gregarious phase of *Schistocerca gregaria* and other similar swarming locust species (*Locusta migratoria*) are known to exhibit a degree of population-level perceptual and motor lateralization in different contexts (e.g. threat surveillance vs. preferred limb extension). The existence of population-wide lateralization in the solitary phase and whether or not a solitary individual's side biases persist as it gregarizes have not yet been explored. Additionally, the advantages and disadvantages conferred by individual- versus population-level lateralization vary with population density in other invertebrate species, making it a particularly interesting set of traits to examine in *S. gregaria*, which may experience and adapt to both extremes within a single lifetime

To accomplish this, we aim to expand the scope of lateralization assessment in locusts (e.g. conspecific and threat surveillance, virtual Y maze, lateral loom sensitivity), construct behavioural profiles for individual 5th instar solitary locusts, and observe how these individual tendencies shape both the end result and trajectory of gregarization using a combination of repeat lateralization assessment and continuous monitoring (via tagging and tracking in a controlled arena setting) as they undergo gregarization.

PS1.68

Spatial orientation and navigation (Part 1)

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Aquatic gastropod navigation: strategies, sensory afference, and neural-specific gene expression

Aquatic slugs and snails usually rely on water-borne chemicals to find prey. Our recent experiments have revealed several complexities underlying this general statement. Laboratory experiments suggest the pond snail, *Lymnaea stagnalis*, responds to different flow conditions by switching navigational strategies. In stagnant water, we found evidence the snails primarily use chemotaxis, following chemical gradients to find food. In flowing water, the snails were still able to find food, even if the flow was fast enough for turbulent mixing to destroy the chemical gradients. This strongly suggests a switch to chemical-gated rheotaxis, as the snails follow the flow that generates a chemical plume emanating from the food source. Meanwhile, field observations also suggest a strategy switch by the nudibranch, *Hermissenda crassicornis*, in complex, wave-affected flow conditions. We found evidence for chemical-gated rheotaxis when variability in flow direction was relatively low. Remarkably, the slugs also showed evidence of guided movement towards food when variability in the flow direction was high, which could be explained by a switch back to chemotaxis as the extreme levels of mixing created by waves homogenize the chemical cue plumes. Electrophysiological recordings in *L. stagnalis* revealed correspondence between sensory afference and the strength of navigational responses to different food sources, suggesting peripheral processing may be involved in modulating responses. Finally, neuroanatomy based on combining immunohistochemistry and in situ hybridization chain reaction (targeting octopaminergic neurons), also in *L. stagnalis*, revealed unexpected discrepancies between mRNA and protein distributions that complicate mapping of neurotransmitter distributions. In the longer term, we anticipate these projects converging to help understand how peripheral and central neural circuits contribute to the control of navigation behaviour in gastropods.

PS1.35

Social behavior and neuromodulation (Part 1)

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Measuring plasma dehydroepiandrosterone and similar steroids in the male song sparrow

Steroid hormones are key modulators of aggressive behaviour across vertebrates. In many species, male territorial aggression is only expressed in the breeding season and is testosterone dependent. Interestingly, male song sparrows (*Melospiza melodia*) remain aggressive during the non-breeding season despite regressed testes and the lack of circulating testosterone. Dehydroepiandrosterone (DHEA), an adrenal androgen precursor that can be converted to testosterone in the brain, might support non-breeding aggression in male song sparrows. Using radioimmunoassay, high levels of circulating DHEA were found in non-breeding males. However, recent studies using highly specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) found much lower DHEA levels. The overestimation of DHEA by radioimmunoassay might be the result of the antibody cross-reacting with structurally similar DHEA-like steroids. These DHEA-like steroids might modulate non-breeding aggression but remain unexamined in birds. Here, using LC-MS/MS, we measured DHEA, testosterone, androstenedione, pregnenolone, 17?-estradiol, and 11 DHEA-like steroids in the plasma of free-living adult male song sparrows from both the breeding and non-breeding seasons. Subjects were exposed to either a simulated territorial intrusion (STI) or a control treatment (n=8/season/treatment). As expected, testosterone, androstenedione, and 17?-estradiol were detectable in breeding subjects, while DHEA and pregnenolone were not detectable regardless of season. Importantly, the 11 DHEA-like steroids were not detectable in plasma. STI also did not significantly alter plasma steroid concentrations. Therefore, in adult male song sparrows, these 11 DHEA-like steroids are unlikely to be the modulators of non-breeding aggression or sources of radioimmunoassay antibody cross-reactivity.

PS3.10

Motor systems, sensorimotor integration and behavior (Part 3)

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Exploring adaptive locomotor intelligence by altering embodiment in centipedes

Animals exhibit remarkable resilience, maintaining locomotion despite physical damage or environmental changes. This adaptive locomotor intelligence is realized by generating immediate motor strategies based on sensory inputs. While higher centers (e.g., the brain) generally handle global strategies like direction and speed, lower-level decentralized networks, such as central pattern generators (CPGs), manage autonomous coordination of the body and limbs via sensory feedback. However, how these systems are integrated to produce adaptive and resilient behavior remains insufficiently understood. To address this issue, this study focuses on the exploratory locomotor behavior of centipedes. Despite lacking sophisticated vision, centipedes navigate complex terrains by integrating decentralized leg control with active sensing, which involves utilizing their antennae and anterior trunk to explore the environment. This makes them a suitable model for studying the interaction between high-level decision making and low-level locomotor control. Building on previous modeling studies that reproduced versatile locomotion of intact centipedes, this study further investigates the inherent control mechanisms for situation-dependent locomotion by artificially altering the centipedes' embodiment. Behavioral observations were performed on individuals subjected to amputation of the legs and antennae, thereby modifying their morphology and sensory inputs. In this presentation, I would like to report on the observed behavioral changes based on kinematic analyses and discuss control mechanisms that enable them.

PS1.33

Social behavior and neuromodulation (Part 1)

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Dopaminergic regulation of decision-making about water stress in desert harvester ants

Our project examines how social animals make decisions about the risk of environmental stress. The red harvester ant, *Pogonomyrmex barbatus*, lives in the deserts of the southwestern United States, where ants must spend water to gain water. Foragers lose water to evaporation while searching for seeds, which provide both nutrition and fat that can be metabolically converted to water. The food they retrieve is shared with the rest of the colony. As climate change brings hotter and drier conditions, colonies face increasing risk of water loss during foraging. An outgoing forager is stimulated to leave the humid nest by its rate of olfactory encounters with returning nestmates carrying seeds. A forager's decision also integrates its recent experience of current dryness on previous trips. Colonies show consistent differences in foraging decisions under dry conditions. Risk-averse (RA)

colonies reduce foraging to conserve water, sacrificing food intake, whereas risk-tolerant (RT) colonies continue foraging despite the high physiological cost. Previous transcriptomic and pharmacological studies suggest that dopamine (DA) mediates forager decisions about the risk of water loss. We hypothesize that differences in foraging strategy are associated with differences in dopaminergic circuits in the brain. Using immunohistochemistry, we labeled dopaminergic neurons (DANs) and visualized candidate dopaminergic circuits in RA and RT colonies. Based on conserved brain organization and homologous DAN clusters identified in *Drosophila melanogaster*, we identified candidate dopaminergic clusters that may regulate dopamine release during foraging decision-making. These include PAM1, with cell bodies near the antennal lobes and projections to the mushroom bodies (MBs), and PPL1 and PPM3, both associated with the MBs and projecting to the central complex. Comparing the neuroanatomy of DAN clusters in RA and RT colonies will help reveal how variation in dopaminergic circuits contributes to differences in foraging strategy under environmental stress.

PS2.60

Auditory systems and acoustic signaling (Part 2)

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Prevalent mismatch between behavioral and physiological audiograms across mammals

Behavioral and physiological audiograms are widely used to assess auditory sensitivity in animals. Many studies have compared the differences between the two types of measurements, but they were often overlooked in practice, with physiological audiograms frequently used as direct proxies for perceptual hearing sensitivity. Interpretation of these measures and the sources of their divergence remain poor. In this work, we measured behavioral audiograms in Pratt's roundleaf bat (*Hipposideros pratti*) using a two-alternative forced-choice (2AFC) paradigm, and obtained physiological audiograms using auditory brainstem responses (ABR) and inferior colliculus (IC) evoked potentials. We found striking mismatches between behavioral and physiological audiograms, particularly near the peak frequency of echolocation calls. We further examined the effect of body temperature on ABR audiograms in *H. pratti*, *Hipposideros armiger*, and *Eptesicus serotinus*. We demonstrated that ambient temperature regulation is insufficient to stably maintain body temperature in anesthetized bats. Reduced body temperature significantly elevated ABR thresholds by up to ~20 dB near peak and end frequencies, and this effect was consistent across species. Additionally, we compiled published audiograms from bats and other mammals, obtaining both behavioral and physiological audiograms for 10 bat species and 15 other mammalian species. Comparative analysis revealed that mismatches between behavioral and physiological audiograms are widespread in mammals. Our findings highlight the importance of body temperature monitoring during physiological recordings and caution against directly equating physiological thresholds with perceptual sensitivity.

PS2.97

Metabolism, biological rhythms and homeostasis

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Conjugated control: redefining steroid hormone regulation in mosquito reproduction

Ecdysone is essential for mosquito reproduction, namely blood meal-induced oogenesis in *Aedes aegypti*. The prevailing model posits that a vertebrate blood meal stimulates ovarian ecdysone synthesis, followed by free diffusion of ecdysone through the hemolymph and passive uptake into the fat body to activate transcription of yolk proteins. This view has been challenged by recent data showing that specific organic anion transporting polypeptides (OATPs) are required for all ecdysone biology and for steroid hormone uptake into cells (Okamoto et al., 2018; Hun et al., 2022). In this work, we combine biochemical, structural, and physiological approaches to define steroid hormone chemistry and transport during mosquito reproduction. Focusing on an *Aedes aegypti* OATP essential for successful reproduction, our preliminary data indicate that steroid uptake requires a negatively-charged conjugated moiety. We therefore hypothesize that a conjugated steroid hormone, rather than free ecdysone, is the transported and physiologically relevant hormone. Identifying this endogenous conjugated substrate and elucidating its OATP-mediated uptake at molecular and organismal levels will clarify how a systemically circulating hormone achieves tissue- and time-specific signaling in *Aedes aegypti*.

PS3.6

Motor systems, sensorimotor integration and behavior (Part 3)

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A causal neuromechanical model of voice production in songbirds

Songbird vocal learning is a powerful model system for studying motor learning, but how neural signals are transformed into sound remains poorly understood. Recent developments in biologically-inspired robotics and artificial intelligence strongly emphasize the need for a systems-view on motor control, termed embodied motor control, where interpreting the activity of neural circuitry is best understood by including the biomechanics of sound generation, muscles, and the exterior world.

Here we present a physics-based continuum model for voice production and motor control in the songbird vocal organ, the syrinx. We implemented a fully articulated, Fluid-Structure-Acoustic Interaction (FSAI) model of the adult zebra finch syrinx and vocal tract, including anatomically correct 3D geometry of muscles and vibrating tissues. We tested model predictions against experimental data in three tiers. Firstly, we predict dynamic muscle force from naturalistic motor neuron activity using our open-source muscle model, JiSuJi. Secondly, we tested predictions of syrinx posturing due to individual muscle recruitment against 3D motion of the syrinx skeleton in vitro. Finally, we tested predictions of the acoustic model - from vibrating FSAI tissues to the sound radiated at the beak when driven by various pressure and muscle recruitment patterns against in vitro experiments. Our model successfully predicts the effects of muscle recruitment on both posture and acoustics.

We present a systems-level framework that causally links neural activity, biomechanics, and acoustic output, which is essential when realistic representations of voice physiology are required, as in motor control studies or clinical voice management. Embodied models of virtual animals or organs aid in understanding the motor control of subtle, high-dimensional movements that constitute natural behavior, and enhance moves to reduce, refine, and replace animal use.

PS1.55

Social behavior and neuromodulation (Part 1)

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Complementary use of CF and FM components during prey localization in horseshoe bats

Echolocation in horseshoe bats is based on a unique CF-FM signal design that combines a constant-frequency (CF) component with a frequency-modulated (FM) component. Whereas FM signals are widely used in many bat species and suited for object localization, CF signals are considered particularly suited for detecting fluttering prey. In this study, we investigated the respective roles of CF and FM components in prey localization. Pulse emission direction was recorded using a microphone array, while bat and prey positions were tracked with a motion capture system to quantify prey localization accuracy. During the attack sequence following prey detection, we applied band-limited noise targeting the CF component, the FM component, or a control frequency band, and analyzed the resulting changes in emission accuracy and capture behavior. Masking the CF component caused the pulse emission direction to deviate from the prey and resulted in the complete abandonment of capture attempts. In contrast, when the FM component was masked, bats continued to direct their pulses toward the prey and still attempted capture. However, under FM masking, flight speed decreased as bats approached the prey, hovering behavior increased, and capture time was prolonged compared to control conditions in which noise was presented outside the relevant frequency bands. These results indicate that glint information reflected in the CF component, generated by fluttering prey, functions not only as a detection cue but also as a directional localization cue during the subsequent approach phase. In contrast, during the final capture phase, when precise ranging becomes increasingly critical, the FM component provides essential distance information. This functional dissociation demonstrates a complementary utilization strategy in which CF and FM components are adaptively engaged according to behavioral context.

PS3.22

Motor systems, sensorimotor integration and behavior (Part 3)

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Locomotion mechanism of train millipedes in diverse environments: Laboratory experiment and mathematical modeling of wavy synchronization

Biological structures have inspired the development of technologies such as snake-shaped and centipede-shaped robots. From preliminary experiments, we observed that train millipedes (*Parafontaria laminata armigera*) maintain a straight trunk without significant posture fluctuation while they cooperatively control multiple legs and shows wavy synchronization. This study aims to quantify these leg movements on three different floor

materials by using DeepLabCut, and propose a mathematical model that reproduces the observed empirical data.

First, we recorded and analyzed the walking pattern of actual millipedes. The millipedes were captured in Nagano Prefecture, Japan. The movements were recorded from the side using a video camera on floors with different friction levels. Multiple body parts, including each leg tip, the head and tail, were tracked by DeepLabCut, a deep-learning-based software.

Next, we proposed a mathematical model based on a phase oscillator model to reproduce the empirical data. Due to the simplicity of the phase oscillator framework, we could easily estimate the parameters.

In our presentation, we will present the quantitative results of the gait patterns obtained from the experiments on different surfaces and discuss the mechanisms of adaptive locomotion revealed through our experimental data and mathematical modeling.

PS2.13

Motor systems, sensorimotor integration and behavior (Part 2)

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Quantifying behaviour as a gateway to understanding the bumblebee brain and the neuroethological effects of pesticides

Bumblebees (*Bombus terrestris*) are central place foragers that must forage not only to support themselves, but also to support their numerous hive-mates. As soon as a foraging bumblebee leaves the hive, it must familiarise itself with its surroundings, to find the usually inconspicuous hive entrance on its return. This initial exploration behaviour, coupled with path integration-based vector navigation makes bumblebees excellent way-finders. However, as pollinators, bumblebees come in frequent contact with pesticides. While not intended to harm them, these pesticides have detrimental effects even at sublethal doses. Detriments comprise an array of motor and sensory deficits, including reduced foraging efficiency, which was linked to impaired navigational abilities. Compared to motor and sensory defects, such high-level cognitive impairments are poorly understood. To investigate the behavioural strategies bumblebees use during their initial exploration of a new environment, and the effects pesticides might have on that, we developed a state-of-the-art setup consisting of a large behavioural arena and a novel lock-on tracking camera system. This setup exploits our recent finding that bumblebees perform vector navigation when walking. Using high resolution camera tracking, individual bumblebees were filmed during their first trip to the behavioural arena. Simultaneously, groups of bumblebees were exposed to sublethal doses of three different pesticides: acetamiprid and imidacloprid (two common neonicotinoids), and deltamethrin (a pyrethroid pesticide); and then subjected to the same experimental treatment as non-treated bees. Recordings of walking bumblebees were analysed with a markerless pose-estimation tracking software, tracking body-axis movements, as well as individual limbs. Quantitative analysis of extracted positional coordinates was supplemented with qualitative analysis of behavioural patterns. These data yielded comprehensive, quantitative ethograms of bumblebee behaviour during a free exploration task. These ethograms now form the basis for quantifying the subtle effects of sublethal doses of pesticides on bee behaviour.

PS2.22

Motor systems, sensorimotor integration and behavior (Part 2)

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Effective feeding by a neuromuscular system

Feeding is an essential behavior across species, requiring precise motor coordination adapted to distinct environmental conditions. *C. elegans* feeds on suspended bacteria using a muscular pump, the pharynx, that generates patterned contractions to produce three motor programs: capturing, which traps bacteria at the anterior isthmus; swallowing, which transports them via the posterior isthmus; and chewing, which crushes food for intestinal entry. With its compact, well-annotated neuromuscular system and advanced genetic toolkit, the *C. elegans* pharynx offers a tractable model to study this coordinated behavior. However, the biomechanics of effective feeding remain understudied. Here, we dissect the physical dynamics and neuromuscular basis of the pharyngeal motor program. High-spatiotemporal in vivo imaging and fluid-dynamic modeling confirm that these three programs emerge from a fundamental contraction-relaxation cycle. Effective feeding is determined by fixed subcellular phase relationships between muscle segments, acting upon specialized luminal geometry. To determine the basis of the phase-coupling, we investigated the contributions of electrical and chemical synaptic transmission. Using gap junction mutants, we show that electrical coupling between muscles and supporting marginal cells maintains the phase relationships. In contrast, chemical synaptic transmission is not required, as tonic optogenetic muscle activation restores the motor program after eliminating all neuronal input. Instead, chemical synaptic transmission determines event coupling. The M4 motor neuron regulates the frequency of swallowing but not the phasic relationship between swallowing and other programs. Our work establishes a complete biomechanical description of the *C. elegans* feeding motor program, revealing how cell-cell communication coordinates robust behavior. It provides a foundation for future cellular- and molecular-resolution studies of coordinated behavior in vivo.

PS1.50

Social behavior and neuromodulation (Part 1)

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Midbrain neurons regulate the production of courtship squeaks in female mice.

Across the animal kingdom, context-appropriate vocalizations are crucial for survival and fitness. The periaqueductal grey (PAG), a highly conserved midbrain region, regulates the production of innate vocalizations in vertebrates. How the PAG is organized to regulate different vocalization types (i.e., courtship calls, distress calls, etc.) remains poorly understood. Mice produce two vocalization types: ultrasonic vocalizations (USVs) during social interactions, and distress calls (squeaks) in aversive contexts. The caudal PAG contains specialized neurons that gate the production of USVs, but PAG neurons that regulate the production of squeaks remain to be characterized. To this end, we used chemogenetic silencing and caspase ablation to test the role of different PAG sub-regions and activity-defined PAG neurons in regulating the production of squeaks, in female mice producing squeaks during courtship interactions with males, as well as in males and females exposed to mild footstock. We also used optogenetic activation to test whether neuronal activity in identified PAG neurons is sufficient to elicit the production of squeaks. We show that activation of neurons in the intermediate PAG is sufficient to elicit squeaks, and silencing or ablation of intermediate PAG neurons blocks the production of female courtship squeaks. Notably, these same silencing approaches leave intact the production of footshock-elicited squeaks in females and males. Our findings identify a population of neurons that promotes the production of female courtship squeaks but also challenge the dogma that all innate vocalizations are PAG-dependent. .

PS1.85

Evolution and development

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Neurodevelopment without sensory neurons

Organisms make environmentally informed decisions to survive. This requires the nervous system to integrate external sensory information and intrinsic genetic programs to maintain reliable function while remaining adaptable. Understanding how neural systems achieve this remains a fundamental question in neuroscience: how much is brain wiring intrinsically specified versus shaped by sensory input? While previous studies have focused on specific circuits and brain regions, it remains challenging to disentangle this question across the entire nervous system at single-connection resolution. We address this question by examining how the nematode *C. elegans* connectome develops following early removal of the sensory layer. When sensory neurons are ablated in newly hatched animals, they enter the dauer stage, an alternative developmental program marked by halted ingestion, arrested growth, and reduced movement. In the wild, dauers were induced by environmental stress such as crowding and food scarcity, allowing animals to survive under unfavorable conditions. Surprisingly, despite the removal of almost one-sixth of the neurons in the nervous system, the total number of connections in the sensory-ablated connectome remains comparable to the core wild-type connectome. This suggests that remaining neurons have increased their connections, potentially compensating for the loss of sensory layer. However, only a subset of neurons displayed increased connectivity. This highlights that neurons have different tendencies towards stereotypy and plasticity. Ongoing work aims to correlate these patterns with network motifs and transcriptional signatures. We present the first connectome of a brain developed in the absence of sensory neurons. This dataset provides a unique opportunity to systematically interrogate the tension between stereotypy and plasticity at the whole-brain scale and will serve as a valuable resource for future studies on neural network organization and mechanisms of plasticity.

PS2.9

Motor systems, sensorimotor integration and behavior (Part 2)

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Effects of environmental exposure to estradiol on *Xenopus laevis* vocal behaviors

Estrogenic endocrine disrupting compounds are present at physiologically relevant levels in global surface waters. These compounds can have a significant effect on the development of exposed wildlife and humans and are potentially contributing to the rapid decline of amphibian populations around the world. The African clawed frog (*Xenopus laevis*) is a model organism for the investigation of the effect of estrogenic compounds on sexual behavior because of its well-characterized vocal communication system. Male *X. laevis* produce advertisement calls to attract mates. Previous studies have reported conflicting effects of estradiol (E2) and the synthetic estrogen 17?-ethinylestradiol (EE2) on male *Xenopus* advertisement calling across different species and exposure durations. In this study we aimed to clarify the effects of E2 on *X. laevis* calling behavior over short-term versus long-term exposure. Male *X. laevis* were exposed to E2 (10?? M) and vocal behavior was measured in the presence of a female after 4 days and 30 days of exposure. While the 4-day treatment produced no clear change, trends suggest that the production of advertisement calls decreased over the 30 day period. These findings suggest that environmentally relevant concentrations of E2 may impact the ability of male *X. laevis* to attract a mate. Further research is needed to confirm these results, address the natural roles of estrogens on vocal behaviors, and assess the potential harms caused by endocrine disrupting compounds in the environment.