ABSTRACTS

from

PLENARY LECTURES (PL)

SPECIAL LECTURES (SL)

SYMPOSIA (S)

PARTICIPANTS SYMPOSIA (PS)

and

YOUNG INVESTIGATORS TALKS (YIT)

AUGUST 3

Plenary Lecture 1

Mark Willis (Case Western Reserve University) PL1

"The Neuroethology of odor-guided flight in moths: Interaction of environment, locomotion and sensor structure determines odor-tracking behavior."

Locating important resources from long distances often requires the ability to track odors that have evaporated from their source and been distributed downstream as turbulent dynamically changing distributions of odor molecules known as plumes. Walking and flying animals perform complex maneuvers while tracking odor plumes through very different environmental conditions no matter where their sensors are positioned. Two alternative strategies may allow them to maintain contact with plumes using olfaction: (1) a spatial strategy, i.e., simultaneous comparison of odor concentrations at two locations in space using bilaterally symmetrical sensors, or (2) a temporal strategy, i.e., comparisons of odor concentrations over time. It is thought that animals rapidly navigating in three-D through turbulent odor plumes use a temporal strategy, while animals navigating slowly near surfaces use a spatial strategy. However, animals can move at a range of speeds, change their mode of locomotion, and encounter many environments. Whether an animal tracks plumes spatially or temporally is difficult to determine because the effects of sensory input, behavior, and the environment are entangled.

We have experimentally-manipulated air flow in our laboratory flight tunnel to mimic turbulence experienced by plume tracking insects in different natural environments. These flows and the odor plumes generated by them have been carefully characterized using flow sensors (hot-wire anemometers) and the antennae of the insects we study as bio-detectors. Walking and flying insects tracked plumes in turbulence characteristic of their own natural environment and that of their walking or flying counterpart. Flying moths locate the odor source more often in high than low turbulence environments, and walking cockroaches are equally successful in all but the most turbulent environments. Experiments manipulating the ability of these animals to extract spatial and temporal odor information are ongoing.

Plenary Lecture 2

John Simmers (Université de Bordeaux, CNRS UMR 5227) PL2

"What the spinal cord tells the eyes: gaze-stabilizing eye movements driven by efference copy signals from the locomotory pattern generator in swimming *Xenopus* tadpoles."

All vertebrates, whether running, swimming or flying, are confronted with the effects of their locomotory actions on the ability to perceive their surrounding environment. A possible consequence of self-generated body motion is head movement that cause retinal image drift with a resultant degradation of visual information processing. In order to maintain visual field acuity, retinal image displacement is counteracted by dynamic compensatory eye and/or head adjustments that derive from the concerted actions of vestibulo-ocular and optokinetic reflexes. We have recently discovered in larval frogs(1) that intrinsic copies of rhythmic locomotor commands produced by central pattern-generating circuitry in the spinal cord are able to drive extraocular motor output patterns that are appropriate for driving compensatory eye movements during undulatory tail-based swimming. Such efference copy signalling, in which the brainstem extraocular nuclei effectively become appropriated to the spinal cord, provides a convenient substrate for driving rapid eye adjustments during locomotion, pre-empting the slower engagement of movement-encoding sensory pathways.

In my talk, I will describe the experimental evidence for this novel interaction between two otherwise functionally and anatomically distinct motor systems and present recent evidence for how the intrinsic locomotory commands are conveyed to their extraocular motor targets and how these feed-forward signals are likely to be integrated with visuo-vestibular feedback information.

(1) Combes D, Le Ray D, Lambert F, Simmers J & Straka H (2008) An intrinsic feed-forward mechanism for vertebrate gaze stabilization. *Curr. Biol.* 18, R241-243.

Symposium 1

"The role of chemosensation in sexual behaviors"

Organizer: **Yehuda Ben-Shahar** (Washington University in St. Louis)

Tainted love: olfactory detection of tetrodotoxin in rough-skinned newts.

S 1.1

Heather Eisthen (Michigan State University)

Tetrodotoxin (TTX) is used as a chemical defense against predation by a variety of organisms, including pufferfish (*Fugu*) and many species of newts; it is also used by some octopus and arrowworms as a venom to subdue prey. TTX is effective in these contexts because it is a potent blocker of voltage-gated sodium channels. Given its often lethal effects, it is perhaps surprising that some of the organisms that produce TTX have also co-opted it for use in intraspecific communication -- for example, it serves as an attractant pheromone in pufferfish. The mechanisms by which TTX is detected and processed as an olfactory stimulus are unknown. We are examining the behavioral significance and neural mechanisms of TTX detection in adult rough-skinned newts, *Taricha granulosa*. Our preliminary data indicate that in a Y-maze with flowing water, adult male newts are attracted to a source of 100 nM TTX, suggesting that the animals can smell TTX and may use it in finding or recognizing conspecifics. Using electro-olfactogram (EOG) recordings, which measure summed generator potentials, we find that TTX evokes robust responses from the olfactory epithelium in concentrations ranging from 1 nM to 1 μM. These responses are similar in shape and time course to those evoked by other odorants, such as whole-body odorants from adult male conspecifics, washings from earthworms, and mixtures of amino acids. In one experiment, we substituted choline chloride for sodium chloride in the Ringer's solution used to bathe the olfactory epithelium during recordings. Interestingly, in the presence of choline chloride, responses evoked by TTX are abolished, but responses evoked by other odorants are unaffected. This result demonstrates that EOG responses evoked by TTX depend on external sodium, suggesting that TTX is transduced by olfactory receptor neurons using a mechanism other than the classical odorant transduction pathway. We are currently pursuing experiments to examine the possibility that TTX detection involves a modified sodium channel in the olfactory epithelium of newts.

Neuronal mechanisms underlying pheromone-mediated sexual behaviour.

S 1.2

Tali Kimchi (Weizmann Institute of Science)

Sexually dimorphic behaviors represent a robust set of innate social and reproductive responses including mating, nursing and aggression. Although theses sexually dimorphic behavioral responses represent the most extreme examples of behavioral variability within a species, the basic principles underlying the sex specificity of brain activity is poorly understood. In rodents, pheromones play a major role in controlling innate sexually dimorphic behaviors, along with substantial neuroendocrine responses. Genetic ablation of TRPC2, an ion channel crucial to the functioning of the vomeronasal organ (VNO) sensory neurons, provides a noninvasive experimental system to directly investigate the role of VNO-mediated pheromone detection in mice. TRPC2 mutant male mice displayed normal mating behavior with an estrous female. However, these mutant males also attempted to mount male intruders instead of attacking them. Recently, I have shown that TRPC2 mutant female mice engage in male-typical courtship and sexual behaviors including mounting, pelvic thrusting and ultrasonic vocalization, indiscriminately toward either a male or a female intruder mouse. Further they display reduction of female-specific behaviors, which include maternal aggression and lactating behavior. These findings demonstrate that VNO signaling plays a critical role in sex discrimination and regulation of sex-specific reproductive behaviors in males and females. We have now developed new experimental methodologies to study pheromone-evoked responses in freely-behaving laboratory and wild-caught mouse colonies, under natural biologically relevant context. I will describe our recent efforts in characterizing novel sexually dimorphic pheromone-induced behavioral and neurochemical responses using ethologically-relevant experimental approaches.

Taste of love: a role for the gustatory sensory system in fly courtship.

S 1.3

Yehuda Ben-Shahar (Washington University in St.Louis)

Sexual behaviors are complex and require sensory inputs from multiple modalities. The majority of the signals, their cognate receptors, and the cells that mediate them are still poorly understood. We use the genetic model Drosophila melanogaster to investigate how animals encode and interpret socially related signals. Here we focus on the role of the gustatory system in mediating sex-related signals in insects. We have identified a novel ligand-gated ion channel (*aguesic*, *agu*) that plays a role in chemosensory functions underlying mating behaviors in Drosophila. *agu* is expressed in a subpopulation of chemosensory bristles on the legs and wings that are distinct from those expressing feeding-related gustatory receptors. Moreover, *agu* is not expressed in the labellum, the primary organ involved in taste, and disrupting agu or inhibiting activity of *agu*-expressing neurons does not alter gustatory responses. Instead, *agu* is sexually dimorphic, and co-expressed with the sex-determination gene fruitless in adult legs. Consistent with this pattern, blocking *agu*-positive neurons or mutating the *agu* gene delays the initiation and reduced the intensity of male courtship. These data indicate that *agu* and the cells expressing it are an essential component of the peripheral sensory system that determines sexual behavior in Drosophila. In addition, our results indicate the presence of at least two types of chemosensory bristles on appendages, some specialized to influence mating and some for feeding.

Chemosensory contributions to moth pheromone-mediated behaviour.

S 1.4

Neil Vickers (University of Utah)

For many insects odors play a vital role in mediating communication between sexes. One of the best-studied interactions involving chemosensation concerns the long distance odor-mediated attraction between male and female moths. Olfaction shapes every facet of this behavior: males respond to intersection with packets (or filaments) of pheromone-laden air by surging upwind and then relax into crosswind, casting flight in clean air. Males are highly sensitive to female pheromone blends and readily discriminate conspecific blends from those released by females of other species - even if the difference is the presence or absence of a single odorant. Decisions and discriminations are made while on the wing. These behavioral insights have formed the framework for experiments designed to further our understanding of the olfactory system - how it is organized to detect, discriminate and process odor information. Our studies build upon the excellent knowledge base established by other groups and take an evolutionary perspective by utilizing a group of closely-related moths. I will describe experimental results from a variety of different experimental approaches that are providing new information about chemosensation in moths.

Symposium 2

"Neural information processing in cephalopod cognition"

Organizer: **Frank Grasso** (Brooklyn College)

Evolution of brain complexity: Learning and memory in chambered *Nautilus*.

S2.1

Jennifer Basil (City University of New York)

We study learning in the ancient cephalopod *Nautilus pompilius* as a way of understanding how its simple brain and behaviours may have evolved in its more derived relatives, the coleoid cephalopods (octopuses, cuttlefish and squid), whose brains are heavily invested in learning and memory. Nautilus retains a primitive brain and expresses a limited behavioural repertoire, whereas coleoid brains are among the most complex of all the invertebrates, containing several discrete lobes dedicated to learning and memory storage, the vertical and frontal lobe complexes. The nautilus CNS lacks known homologues or analogues of these regions, and instead the brain probably approximates those of the ancestors of both extant subclasses. Nautilus is therefore a uniquely placed genus for studies of the evolution of neural structures supporting memory. Previously we found temporally separated STM and LTM expression in Nautilus, similar to results from coleoids, and stable LTM expression for at least three weeks post-training, also comparable to results from the other, more derived cephalopods. Because the specialised structures in the coleoid CNS would evolve only under sustained directional selection, we sought to identify similarities and differences in learning ability in nautilus through behavioural assays with intact, free-moving animals. By providing increasingly complex cue arrays to be learned for spatial navigation, we investigated whether nautilus would perform poorly in a complex cognitive task compared to Coleoids. Nautiluses were trained to escape from a shallow, brightly lit arena into deep, dark water, with the aid of multiple visual cues located proximate and distant from the escape point. We found evidence for rapid learning, long memory, and dynamic switching between navigational strategies when the cue arrays were changed. This suggests that despite lacking the dedicated neural structures of its more derived relatives, Nautilus performs surprisingly well at simple cognitive tasks. Perhaps the antecedent of the vertical or frontal lobe may be present in the Nautilus CNS, and may be identified through future electrophysiological experiments.

Behavioural and neural evidence for separate visual memory systems in embryos and juveniles of cuttlefish (*Sepia officinalis)*. S 2.2

Ludovic Dickel (University of Caen Basse-Normandy)

In juvenile cuttlefish, memory abilities seem to mature gradually during development. Very short term memory processes involved in prey-pursuit behaviour develop early in life (within the first few days after hatching) while long term retention performance of an associative learning task (learned inhibition of the predatory behaviour) increase throughout the first three months of life. These phenomena are correlated with the post-embryonic maturation of the vertical lobe complex, a highly associative brain structure in cephalopods. Paradoxically cuttlefish will prefer, for days, to feed on prey to which they were familiarized at early stages of development (*in ovo* or just after hatching). These recent data suggest the existence of food imprinting in early Sepia juveniles. Putative neural basis and adaptive advantages of such early visual memory abilities will be discussed.

Coordination of forceful and fine manipulation by suckers and arms *Octopus*

S 2.3

Frank W. Grasso (Brooklyn College, CUNY)

Octopus arms are versatile appendages that render the octopus capable of coordinated fine and forceful manipulation of objects: unscrewing jar lids in the laboratory and retrieving live crabs from deep rocky crevices in the field are examples that are well documented. The biomechanics of octopus arms allow them to produce numerous simultaneous bends that function as joints. These may be at arbitrary locations along and at arbitrary angles relative to the long axis of an arm. And the arm sections between these joints may reshape themselves independently. While the uniqueness of this mode of manipulation has long been appreciated, the contributions of the approximately 300 suckers on each arm in the natural behavior of these animals have received lesser attention. Each sucker functions as a fine-manipulation device that can attach to an object or surface with great adhesive force. Once attached, powerful muscles within the sucker permit translation of the object to arbitrary points within a hemispherical space around the sucker's anchor position on the arm without the loss of attachment. Suckers coordinate attachment patterns and movement between suckers, both with and without loads, and can pass items between suckers. Our studies of arm-sucker coordination reveal that the sophisticated intersucker coordination patterns are complimented by sensory-motor integration amongst the numerous effectors and sensor components of the arm as well. Contrary to a view of suckers as passive agents reflexively reacting to surface contact, our results demonstrate that suckers anticipate surface contact, and inform the movements of other suckers as well as the arm. I will discuss the ways in which these results are consistent with the known neural organization of the octopus nervous system and what they tell us about multiple levels sensory-motor feedback distributed between at least three levels of ganglionic organization: sucker, brachial ganglia and cerebral ganglia.

What can cephalopods teach us about the organization of cognitive neural networks?

S 2.4

Binyamin Hochner (Hebrew University)

Like the mushroom bodies of insects, the cephalopod vertical lobe (VL) reveals an architecture characterized by parallel and stratified arrangements of many neurons with *en passant* innervation. The work pioneered by JZ Young and MJ Wells confirmed the involvement of the VL in the highly sophisticated behaviors of modern cephalopods. We therefore believe that understanding the neurobiological properties of these unique invertebrate structures will advance our understanding of the principles enabling neural networks to control complex behaviors. Results from our research on VL connectivity and implementation of this connectivity in learning and memory circuitry demonstrate the fruitfulness of this approach. I will further discuss the striking differences between short- and long-term synaptic plasticity in the VLs of *Octopus vulgaris* and *Sepia officinalis* that make these animals uniquely interesting for investigating how neural networks subserve complex behavior.

Symposium 3

"Novelty processing in neurons and large-scale neural populations to build auditory objects: evidence from animals and humans"

Organizers: **Manuel S. Malmierca,** University of Salamanca and **Carles Escera,** University of Barcelona

Stimulus-Specific Adaptation in Single Neurons: Effect of Stimulus Type and Degree of Neuronal Specialization. S 3.1

Ellen Covey and **Jessica Koch**, (University of Washington)

Previous studies have shown that the auditory midbrain of vertebrates contains neurons that decrease firing in response to a repeated stimulus but respond fully whenever a different sound occurs. This phenomenon is called stimulus-specific adaptation (SSA), and is thought to be the basis for detection of novel sounds. Neurons in the mammalian inferior colliculus (IC) are functionally heterogeneous and include specialized spectrotemporal pattern detectors as well as unspecialized neurons that respond to any sound that enters their frequency response area. Specialized neuronal types have been extensively studied in echolocating bats, and include neurons that only respond to downward or upward frequency sweeps, sinusoidally frequency modulated tones, or tones of a specific duration. Although the bat IC does contain neurons with a high degree of SSA, our initial survey of the awake bat IC showed that the amount of SSA is less than in the anesthetized rat preparations studied previously. One possible explanation is that SSA is stronger during anesthesia and/or sleep than during waking. Another possibility is that the bat IC contains a higher proportion of specialized neurons and that these neurons do not exhibit SSA. To test the second hypothesis, we recorded from neurons throughout the IC and found that none of the pattern-selective neuron types exhibit SSA, nor do "frequency filter" neurons that are narrowly tuned to the 28 kHz quasi-constant frequency range used by the bat while searching for prey. We did find a positive correlation between breadth of frequency tuning and amount of SSA, with filter neurons at one extreme and broadly responsive "novelty detector" neurons at the other. Because bats emit repetitive sounds during echolocation, especially during the search phase of foraging, it would likely be counter-productive for neurons to adapt to these specialized sounds, so the bat may be an unusual in that there is little adaptation in the echolocation system.

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Functional mechanisms that mediate stimulus-specific adaptation subcortically.

S 3.2

Manuel Malmierca, David Pérez-González, Flora Antunes, Olga, Hernández (INCYL, Univ. Salamanca), **Israel Nelken** (The Alexander Silberman Institute of Life Sciences) & **Ellen Covey** (Univ. Washington, Seattle)

Neuronal adaptation is an important property of the nervous system and a special form of it is stimulus-specific adaptation (SSA). SSA has been shown to occur at the auditory cortex (AC) and inferior colliculus (IC) levels. We will review the relation between SSA and the detection of novel sounds. We recorded the responses of single neurons in the IC and auditory thalamus (MGB) of rats to an oddball condition, i.e., an infrequent (novel) sound that occurred randomly in a sequence of repetitive (standard) stimuli, both stimuli being pure tones of different frequencies in a similar way as used in the mismatch negativity (MMN) studies. The data indicates that the majority of neurons in the IC and MGB respond more strongly to a novel stimulus than to one that is predictable, i.e., they show strong SSA. Additional experiments blocking GABAergic inhibition show that SSA is reduced during Gabazine (a GABA-A blocker) application in the IC. These findings altogether suggests that SSA is created at lest in part at the IC and MGB in a bottom-up process and GABAergic inhibition in the IC may play a role in the generation of mismatch negativity (MMN) evoked by novel stimuli.

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Novelty detection and aging in the medial geniculate body: $GABA_A$ receptors and aging S 3.3

Donald M. Caspary and **Benjamin D. Richardson** (Southern Illinois University School of Medicine)

A number of recent studies suggest that novelty detection/detection of deviant stimuli in the auditory thalamus, the medial geniculate body (MGB), may be determined, either directly or indirectly, by its inhibitory inputs. Significant GABAergic inhibitory inputs to the MGB from the thalamic reticular nucleus (TRN) and those ascending from the inferior colliculus (IC) have been suggested to be involved in deviance detection at the thalamic level (Yu et al.,2009; Malmierca et al., 2009). Neurons in sensory thalamus are unique in that they contain $GABA_A$ receptors ($GABA_A$Rs) with different subunit constructs, locations and function. Wild-type $GABA_A$ receptors ($2\alpha_1 2\beta_2\gamma$) are located postsynaptically and mediate fast transient inhibition while $GABA_A$Rs containing α_4 & δ subunits are located extrasynaptically and mediate tonic inhibition. In humans, impaired ability to clearly identify novel/deviant stimuli in a complex acoustic background is one consequence of aging. Age-related changes in the nature of $GABA_A$ receptors has been shown to occur in several different central auditory structures resulting in altered coding of acoustic information in aged animals (Caspary et al., 2009). Therefore, age-related changes in the nature of GABA inhibition could be expected to alter the ability to identify novel stimuli as well. Preliminary studies suggest that both of these receptor types are present in the MGB and may be impacted by aging. *In vivo* recordings in rat MGB reveal enhanced and/or suppressed responses to deviant stimuli using a number of different stimulus paradigms. *In vitro* patch clamp recordings, neurochemical and bindings studies show the presence of synaptic and extrasynaptic $GABA_A$Rs in MGB. We will speculate on the role of synaptic and extrasynaptic $GABA_A$Rs in MGB while addressing the possible role for these sub-types in mediating deviance detection. The impact of aging on these receptors and the nature of the deviance detection response will be discussed.

On the laminar origin of stimulus specific adaptation in auditory cortex.

S 3.4

Jan W. H. Schnupp, José García Lázaro & Francois D. Szymanski (Oxford University)

Neurons in primary auditory cortex (A1) are known to exhibit a phenomenon known as stimulus specific adaptation (SSA). When tested with pure tones, they will respond more strongly to a particular frequency if it is presented as a rare, unexpected 'oddball' stimulus, than when the same stimulus forms part of a series of common, 'standard' stimuli. While SSA has been observed in the subcortical paralemniscal auditory pathway, it is thought to be weaker and rarer among neurons of the lemniscal pathway which provide the main afferent input to A1, so that SSA seen in A1 is likely generated within A1 by local mechanisms. In order to investigate the contributions that neural processing within the different cytoarchitectonic layers of A1 may make, we simultaneously recorded multi-unit activity and current source density profiles across all layers in A1 of the rat in response to standard and oddball tones. Current source density is thought to reflect mostly the net input currents to neurons at a given depth, while the multiunit activity reflects their average output. While our results show that SSA can be observed throughout all layers of A1, right from the earliest part of the response, there are nevertheless significant differences between layers, with SSA becoming significantly stronger as stimulus related activity passes from the main thalamorecipient layers III and IV to layer II/III. This suggests that neural activity in the supragranular layers may make a particularly strong contribution to the emergence of SSA.

Primitive sensory intelligence in the rat brain as measured by mismatch negativity like local field potentials. S 3.5

Timo Ruusuvirta (University of Turku)

There has been a challenge to probe into neural representations of perceptual attributes of different complexities in the brains in different animal species and during different behavioral states. We have applied the mismatch response (MMR, an analogy of the mismatch negativity in human adults) of local field potentials (LFPs) of the brain to meet this challenge in the auditory modality. Our research in urethane anesthetized rats has discovered the abilities of these animals to represent physical sound features (frequency), combinations of two of such features (frequency and intensity), as well as abstract relationships between sound feature levels across sequential sounds (melodic orderings of the sounds). Ongoing MMR studies capitalizing on this research has extended the auditory attributes addressed to those known to be critical in language acquisition, such as the segmentation of serial linguistic and non-linguistic auditory material and the extraction of abstract (grammatic) rules from such material. In all, the research aims at paving way to the understanding of our higher-order auditory cognition in the framework of comparative studies and across different levels of analysis, from molecular to systems.

Symposium 4

"Neuroeconomics and decision in small neuronal circuits"

Organizer: **Rhanor Gillette** (University of Illinois at Urbana-Champaign)

The Neuroethology of primate social decision making. S 4.1

Karli Watson (Duke University)

In order to navigate their way through a dynamic social environment, human and nonhuman primates alike must identify salient social signals, assign reward or punishment value to those signals, and adjust their behavior appropriately. In the first part of my talk, I will discuss my findings regarding the involvement of the primate orbitofrontal cortex (OFC) in representing social and non-social rewards in a simple decision making task. Neurons in the OFC respond to food and liquid rewards, and lesions of this region interfere with social behavior and decision making. I found that individual neurons in the macaque monkey OFC represent both gustatory and social rewards, and do so in a way that matches monkeys' relative preferences amongst outcomes. These observations are consistent with the idea that OFC integrates multiple sources of information to compute a common metric of value guiding decisions. In the second part of my talk, I discuss how genetic polymorphisms that regulate serotonin signaling influence individual behavioral variation in response to social stimuli. Specifically, monkeys carrying a copy of the 5-HTTLPR "short" allele, which in humans is associated with stronger amygdala response to social threat, show greater sympathetic arousal and increased avoidance behavior in response to social threats. Such behavioral signatures are also seen in humans, and in extreme cases can manifest as psychopathology. My results suggest that variations in social behavior, far from being merely pathological, confers adaptive benefits in complex social societies.

Multiple mechanisms of behavioral choice in the leech. S 4.2

William Kristan (University of California at San Diego)

Historically, behavioral choice has been thought to be a competition among "command modules", groups of neurons that individually elicit specific behaviors. We have looked for such interactions among neuronal circuits that produce feeding, swimming, crawling, local bending, and shortening in medicinal leeches. We have looked for these interactions by stimulating neurons that elicit one behavior while the leech is producing another one. We have found several different neuronal mechanisms—some very subtle and others exceedingly strong—that choose which behavior is produced, but none of them involve competition among command modules. The evolutionary causes (and consequences) of these different mechanisms could determine which mechanism is used in any particular case.

Modulatory neurons and behavioral sequences. S 4.3

Jian Jing (Mt Sinai School of Medicine)

One hallmark of animal's behavioral expression is organization of behaviors in sequences, based on interactions among sensation, experience and internal states. Molluscan circuitry studies provide insights on how modulatory neurons contribute to the emergence of behavioral sequences by altering internal states. We describe two examples in *Aplysia*. (1) During the motivational shift from hunger to satiety, animals initially rigorously feed on food, but may reject food and eventually avoid it as animals become satiated, which is mediated partly by modulatory neurons with *Aplysia* neuropeptide Y (apNPY). apNPY injection shortens meal size. apNPY neurons are localized in a gut-innervating nerve that transmits information about satiation. Stimulation of this nerve, which results in apNPY release, and apNPY application convert ingestive motor programs to egestive ones. Thus, peptide reconfiguration of the feeding network contributes to behavioral sequences due to changes in motivation states. (2) Behavioral activity level critically depends on the arousal state, which is mediated partly by multifunctional serotonergic interneurons. These interneurons are activated broadly by alerting/noxious stimuli, and promote activity in a variety of neural networks including locomotion and feeding. Significantly, the specific actions in these networks are distinct. In the locomotor network, these interneurons directly activate locomotion so the animals can escape from the potential danger. However, in the feeding network, they persistently but indirectly act by promoting activity in a serotonergic modulatory neuron of feeding network, which promotes feeding but do not initiate feeding directly. Thus, direct and conditional activation of two neural networks allows the serotonergic interneurons to enable behavioral transition from escape locomotion to feeding (when food becomes available). In summary, specific classes of modulatory neurons are essential mediators of behavioral sequences.

Value, risk, reward and decision in a simple nervous system. S 4.4

Rhanor Gillette (University of Illinois)

Appetitive state manifests in the excitation state of a goal-directed neuronal network, sets sensory thresholds for behavioral output and biases an effector network between alternative outputs in the predatory sea-slug *Pleurobranchaea californica*. Appetitive state is conserved in the isolated CNS in terms of feeding network excitation state and decisions between fictive avoidance and orienting. These decisions are reversibly controllable through manipulating the excitatory state of the feeding network. Corollary outputs from the feeding network target specific neurons of the turn network to bias the symmetry of the turn network output response to sensory input from avoidance to orienting. The simple neuronal scheme relating appetitive state and approach/avoidance decision in this model system may constitute a core of both simple and complex economic decision-making across phyla. They also provide basis for autonomous cost-benefit decision in artificial-life modeling and robotics.

Support by NSF IBN 0843621 and NIH R21 DA023445.

Symposium 5

"The Knowledge Base of Insect Navigation"

Organizer: **Jochen Zeil** (The Australian National University)

Limits of animal navigation: a theoretical perspective. S 5.1

Allen Cheung & **Tobias Merkle** (The University of Queensland)

In principle, there are two strategies for navigating a straight course. One is to use an external directional reference and continually reorienting with reference to it, while the other is to infer body rotations from internal sensory information only. It turns out that while the first strategy will enable an animal or mobile agent to move arbitrarily far away from its starting point, the second strategy will not do so, even after an infinite number of steps. There is a fundamental limit to any type of navigation (including path integration) relying purely on idiothetic cues. To compound the problem, the second strategy rapidly leads to an area of positional uncertainty greater than would result from a pure random walk. Thus, an external directional reference—some form of compass—is indispensable for ensuring progress away from a point of departure (like a nest). This limitation must place significant constraints on the evolution of biological navigation systems. I will briefly mention some different examples of external cues which, from a navigation perspective, may be considered as “compasses”.

View-based navigation in ants. S 5.2

Antoine Wystrach (BBE Macquarie University / CRCA University of Toulouse)

In a recent paper in Current Biology, Wystrach and collaborator have for the first time adapted an experimental paradigm to study insect navigation that was traditionally used to investigate the neural representation of space in vertebrates with the aim of disentangling the role of global and local cues in view-based navigation in animals. Their work now suggests that navigational abilities can be explained in a much simpler way than hitherto thought in the experimental psychology literature.

The role of colour vision in nocturnal homing. S 5.3

Hema Somanathan (Indian Institute of Science Education and Research, Trivandrum), **Eric Warrant** (Lund University), **Renee Borges** (Indian Institute of Science, Bangalore) & **Almut Kelber** (Lund University)

Bees are trichromatic and possess photoreceptors with peak sensitivities in the UV, blue and green region. Diurnal colour vision in honeybees has been known for a century, while later studies have shown that honeybee colour vision rapidly deteriorates after sunset. Most bees are colour-blind under moonlit conditions and cease to fly even under conditions brighter than starlight. All bees possess apposition compound eyes that are typically found in diurnal insects. The first evidence for nocturnal colour vision in an animal with apposition eyes has been provided by us in experiments on the nocturnal Indian carpenter bee *Xylocopa tranquebarica*. *X. tranquebarica* possesses special visual adaptations for dim-light and uses colour vision to discriminate artificial landmarks at the nest in starlight. This finding obtained using homing bees under natural illumination is remarkable because their insensitive apposition optics were believed to be incapable of colour vision. Our experiments also show that nest odours do not play a role in homing in these bees that tend to rely on visual landmarks for finding their nests in the dark.

The overlap region of navigation and dance communication in honeybees.

S 5.4

Rodrigo J. De Marco (Max Planck Institute for Medical Research, Heidelberg)

One of the most sophisticated forms of cooperative foraging occurs in a social insect that has reached a peak of social organization, the honeybee. Honeybees cooperate by sharing newly discovered sources of food. They are famous for a behaviour called the waggle dance, which they use to recruit conspecifics to rich food sources. The key stimulus for dancing is the presence of food at a given feeding site, and the higher the food availability at the site the more likely a forager will dance and the greater the strength of such behaviour. Thus, each forager establishes a flow of energy and information from the source to the colony. This is why the dance is referred to as a striking organizational behaviour. A honeybee's dance for a given source of food typically leads to an increase of inspections by other colony members in the surroundings of that source, a phenomenon referred to as a local recruitment. Yet, the rates of local recruitment achievable through dances are much lower than expected if honeybees were to respond to such dances by flying directly towards the indicated goals. This has puzzled biologists for decades, and demands considering three critical aspects of communication and navigation in honeybees. First, the amount of redundancy involved in the dance signals remains unknown. Second, on the receiver side communication means computing a 'stored variant' of a symbolic entity selected and transmitted by a sender. Third, navigation is not invariant to past experience. It follows, therefore, that a deeper understanding of how a dancer's behaviour is mapped to that of its followers lies well within the overlap region of communication and navigation, particularly with respect to the navigational memories that are shared -or not shared- by both dancers and followers. I will argue that honeybees respond to the dances of other foragers by inspecting the food sources they are already familiar with, irrespective of the goals being signalled by the current dances. They inspect their past food sources first in response to the dances of other foragers. If food is absent at such goals, they will follow additional dances, also extending their searching times and foraging areas selectively. In doing so, they rely heavily on outdoor interactions with other navigating foragers. Eventually, they will find food in the areas of the foragers currently devoted to dancing. This primary response to the dance appears as a key rule of operation in honeybees, and will determine how a colony forages as a unit.

Symposium 6

"Neuroethology of Adaptive Locomotor Responses to the Environment – How simple can it be?"

Organizers: **Matthias Gruhn** (Universitaet zu Koeln) & **Roy E. Ritzmann** (Case Western Reserve University)

Towards wireless monitoring of neural activity during dragonfly prey interception flights. S 6.1

Robert M. Olberg (Union College) & **Anthony Leonardo** (Janelia Farm Research Campus)

In response to an insect passing overhead, the foraging dragonfly takes off on an interception trajectory, aiming a point ahead of the flying prey. If the potential prey deviates in its flight path, the dragonfly corrects its own course so that the bearing to the prey is held constant, a strategy than ensures interception. During the foraging flight the dragonfly adjusts its head angle to maintain the prey's image centered on the dorsal fovea of its compound eye.

Eight pairs of identified neurons are implicated in controlling the dragonfly's flight path as it intercepts its flying prey. These target-selective descending neurons (TSDNs) descend from the brain of the dragonfly to the thoracic ganglia. They show directionally selective responses to small objects moving relative to the dragonfly. Their receptive fields are located in the dorso-frontal quadrant of the visual field, the region that views prey during the foraging flight. Intracellular stimulation of any of these neurons evokes small adjustments in wing position and attitude.

Details of the neural control of prey interception are not obvious. For example, because the dragonfly rotates its head to fixate the prey's image, the signal that indicates the prey's drift is probably very brief. In addition, the dragonfly must factor in its own head angle in determining the bearing of the prey. To more fully understand the neural underpinnings of this complex flight behavior, we are developing the means to monitor TSDN activity from a dragonfly in free flight. Extracellular TSDN activity will be recorded from the mesothoracic ganglion, amplified and transmitted from a lightweight telemetry chip mounted on the dragonfly. Neuronal activity will be correlated with the 3-dimensional trajectories of the dragonfly and its flying prey, reconstructed from high-speed video recordings. Our goal in this approach is to understand how visual information is translated into steering commands for interception in a freely flying insect.

Path simplicity changes inefficient navigation to efficient. S 6.2

John Layne (University of Cincinnati)

Fiddler crabs are the only animal known to exclusively use idiothetic cues for homing by path integration. It is generally acknowledged that this is the worst possible mode of navigation, in terms of its mathematical potential for errors. However, this only holds if one compares the homing performance of idiothetic path integrators to allothetic path integrators following identical paths. If some simple constraints are placed on the paths traced by idiothetic path integrators – constraints which are more easily adopted by crabs than any other path integrating animals – then idiothetic path integration can out-perform allothetic path integration. These simple constraints turn out to be precisely those found in the paths of foraging fiddler crabs. To demonstrate the relative efficacy of idiothetic path integration, we compared its performance in the face of random measurement error to that of an allothetic path integrating model insect walking the same paths. The paths were acquired empirically from real, foraging fiddler crabs and so embody the constraints in question. Our computer simulations are based on a geometrically correct solution for path integration for the model crab and model insect, and consist of recursive reconstructions, with varying amounts of random noise, of real paths followed by foraging animals. The main difference between crabs and insects in this case is that for crabs it involves integrating three degrees of motion freedom (distances, directions, turns) and for insects it involves integrating only two (distances, directions). We find that the ability to follow a path using three, rather than two, degrees of locomotory freedom vastly improves navigational performance, even of an idiothetic system over an allothetic system.

Cross-modal behavioral algorithms and neural circuits for odor tracking by fruit flies. S 6.3

Mark Frye (HHMI, University of California at Los Angeles)

Flies track fragmented odor plumes through varying visual environments. We show how flight control is mediated by the spatial and temporal distribution of odor cues, how odor influences the gain of optomotor responses, and how these rapid memory-independent interactions require processing by the mushroom body.

Visually guided turning of the stick insect on the slippery surface. S 6.4

Matthias Gruhn (Universitaet zu Koeln)

Walking in stick insects is the result of the coordinated activity of independent pattern generators for each leg and coordinating inter-leg influences. For leg movement adaptations, as they occur during turning or a reversal of walking direction, it is yet largely unknown, how much of the kinematic changes observed in a single leg are the result of neuronal activity in comparison to the role of mechanical interaction between the legs. We have used a slippery surface setup to investigate the role that local neuronal processing in the thoracic ganglia plays in the ability of the animal to show such adaptations to its leg movements. For this purpose, we removed the influence of mechanical interaction between the legs through the ground using a slippery surface and removed sensory input by the successive amputation of neighboring legs. We compared the kinematics of the middle legs of tethered intact animals and reduced preparations mounted above the surface, and analyzed straight walking, turning and backward walking. We then measured the muscle activity and timing of the three major pairs of antagonistic middle leg muscles (protractor/retractor coxae, levator/depressor trochanteris, extensor/flexor tibiae) during forward and backward walking in intact animals and 1- or two-leg preparations. Our results suggest that the single stepping middle leg performs given motor programs on the slippery surface in a fashion that is highly independent not only of mechanical coupling between, but also of the presence of the other legs. Information from neighboring legs, however, appears to be used to shape the latencies of motor output and modify their timing and kinematics in the intact animal.

Plenary Lecture 3

Illusions and illusionists: how to fool the brain with magic and other tricks.

PL3

Susana Martinez-Conde (Barrow Neurological Institute)

All our life, every object we see, every person we know and every incident we experience, are derived from brain processes, and not necessarily the result of an event in the real world. The same neural machinery that interprets the sensory inputs also creates our thoughts, imaginations and dreams; thus the world we experience and the world we imagine have the same physical bases in the brain. Just as physicists study the most minute subatomic particles and the largest galactic conglomerates to understand the universe, neuroscientists must examine the cerebral processes underlying perception to understand our experience of the universe. Visual illusions are one of our most important tools to understand how the brain builds our experience of reality. Likewise, the principles developed by magicians and illusionists throughout history can be very useful to manipulate attention and awareness in the laboratory. Here I will discuss how the visual and cognitive illusions developed by artists and magicians can be applied to the study of the neural bases of consciousness and perception.

AUGUST 4

Young Investigators Talks

Pheromonal and behavioral cues trigger male-to-female aggression in *Drosophila*. YIT 1

<u>María de la Paz Fernández</u>, Yick Bun Chan, (Harvard Medical School), **Joanne Y. Yew** (Temasek Life Sciences Laboratories, Singapore), **Jean-Christophe Billeter** (University of Toronto at Mississauga), **Klaus Dreisewerd** (Westfälische Wilhelms-Universität Münster), **Joel D. Levine** (University of Toronto at Mississauga) & **Edward A. Kravitz** (Harvard Medical School)

Appropriate displays of aggression rely on the ability to recognize potential competitors. As in most species, Drosophila males fight with other males and do not attack females. In insects, sex recognition is strongly dependent on chemosensory communication, mediated by cuticular hydrocarbons acting as pheromones. While the roles of chemical cues in stimulating male to female courtship have been well characterized, the signals that elicit aggression remain unclear. Here we show that when female pheromones or behavior are masculinized, males recognize females as competitors and switch from courtship to aggression. To masculinize female pheromones, a transgene carrying dsRNA for transformer (traIR) was targeted to the pheromone producing cells, the oenocytes. Shortly after copulation males attacked these females, indicating that pheromonal cues can override other sensory cues. Surprisingly, masculinization of female behavior by targeting traIR to the nervous system in an otherwise normal female also was sufficient to trigger male aggression. Simultaneous masculinization of both pheromones and behavior induced a complete switch in the normal male response to a female. In a reciprocal experiment, feminization of the oenocytes and nervous system in males by expression of transformer (traF) elicited high levels of courtship and little or no aggression from control males. Finally, when confronted with flies devoid of pheromones, control males attacked male but not female opponents, suggesting that aggression is not a default behavior in the absence of pheromonal cues. Thus, our results show that masculinization of either pheromones or behavior in females is sufficient to trigger male aggression. Moreover, by manipulating both the pheromonal profile and the fighting patterns displayed by the opponent, male behavioral responses towards males and females can be completely reversed.

Active gaze strategies in two fly species. YIT 2

Barth Geurten, **Roland Kern** & **Martin Egelhaaf** (University of Bielefeld)

In normal life motion vision is always performed under closed loop conditions. The way an animal moves shapes its visual information, which is then analysed and used for motion control. Different movement hence change visual information availability. Flies are known to segregate their movements in to (a) fast rotations of short duration, termed saccades, and (b) intersaccadic intervals of translatory movements and variable duration. This is believed to facilitate the perception of the three-dimensional layout of the environment. The two fly species *Calliphora vicinia* and *Eristalis tenax* have homologous motion sensitive visual interneurons, but exhibit different flight styles. We compared both species' potential neuronal adaptations to the different motion dynamics resulting from these flight styles. To reduce the enormous complexity of the performed flight manoeuvres, we employed clustering algorithms to derive a small set of prototypical movements (PMs). This allows us to segregate the seemingly continuous movements that constitute an animals flight trajectory into a limited number of components, called prototypical movements (PMs). From the transition probabilities between the different PMs we derive a set of rules, i.e. a kind of syntax of the behaviour. As a consequence of the closed-loop nature of the action-perception cycle, each PM leads to characteristic visual input changes. We determined these image shifts on the retina by reconstructing the flight trajectory and arena in virtual reality and by rendering the corresponding distinct optic flow patterns. Even though both species exhibit a similar saccadic flight style, we found the visual input to vary greatly between fly species. In a constrained environment, as for example a small flight arena, *Calliphora* flies at nearly ten times the speed of *Eristalis*. In contrast to *Calliphora* which mainly flies forward, *Eristalis* moves at slow speeds in nearly every possible direction. However, in open space *Eristalis* reaches similar flight velocities to *Calliphora*. To test whether identified homologous visual interneurons are adapted to the specific optic flow encountered by the two fly species, respectively, we presented the reconstructed behaviourally generated optic flow of both species on a high-speed surround LED stimulator (FliMax) to individuals of either species. By using the segregation of flight trajectories into sequences of PMs, we characterised the neuronal response patterns to distinct movement components. The neurons in both species responded similarly to flights of both species, although the response amplitudes of *Eristalis* neurons were larger under both stimulus conditions than *Calliphora*. Currently we are analysing response patterns to pinpoint specific adaptations and their advantages.

The neural basis of a pollinator buffet: floral odor specialization and learning in the moth, *Manduca sexta.* YIT 3

Jeffrey A. Riffell (University of Washington)

Floral traits are thought to reflect specialized adaptations to specific pollinators. Increasing evidence, however, has shown that plant-pollinator interactions are flexible, with tight coevolutionary relationships infrequent among plants and their pollinators. In the Southwest USA the *Manduca sexta* hawkmoth is an important pollinator for many night blooming flowers that exhibit classic "hawkmoth-pollinated" floral traits. *M. sexta*, however, has the ability to learn – by olfactory conditioning – to exploit alternate floral resources when its preferred is no longer present. We therefore examined the neural basis of flower specialization and learning in *M. sexta* using an interdisciplinary approach of chemical analytical determinations, behavioral assays, and multi-channel and intracellular recordings in the moth antennal (olfactory) lobe (AL). Chemical analysis of the floral scents revealed that phylogenetically unrelated flowers pollinated by *M. sexta* were convergent in their odors. By contrast, plants related to the hawkmoth-pollinated flowers, but pollinated by different taxa, had dissimilar odors. AL multi-channel recordings revealed that the neural ensemble represented the hawkmoth floral bouquets similarly. Additionally, behavioral assays demonstrated that naïve hawkmoths perceived the hawkmoth-pollinated floral odors similarly and ignored the other odors. To examine the ability for moths to learn to exploit alternate floral resources, experiments were conducted using simultaneous multi-channel AL recordings while the moth learned to associate a non-hawkmoth floral odor with a reward. Results of these experiments showed that, as the moth learned, the AL neurons increased their firing rate responses and synchronized activity. Further, modulation caused the trained odor representation to become more separable to untrained odors. Together, these experiments demonstrate that spatiotemporal coding in the moth AL transfers floral odor information by two distinct olfactory channels, one involving innate biases and the other through olfactory conditioning.

Circadian and social cues regulate ion channel trafficking in electric fish.

YIT 4

Michael Markham (The University of Texas)

Electric fish generate and sense electric fields for navigation and communication. These signals can be energetically costly to produce and can attract electroreceptive predators. To minimize costs, some nocturnally-active electric fish rapidly boost the power of their signals only at times of high social activity, either as night approaches or in response to social encounters. Here we show that the gymnotiform electric fish *Sternopygus macrurus* rapidly boosts signal amplitude by 40% at night and during social encounters. *S. macrurus* increases signal magnitude through the rapid and selective trafficking of voltage-gated sodium channels into the excitable membranes of its electrogenic cells, a process under the control of pituitary peptide hormones and intracellular second-messenger pathways. *S. macrurus* thus maintains a circadian rhythm in signal amplitude and adapts within minutes to environmental events by increasing signal amplitude through the rapid trafficking of ion channels, a process that directly modifies an ongoing behavior in real time.

First evidence of star orientation in insects. PS I.1

Marie Dacke, Emily Baird (Lund University), **Marcus Byrne** (University of the Witwatersrand) & **Eric Warrant** (Lund University)

Birds and seals are well known to orient to the stars, and we now show that insects also possess this ability. This finding is the result of a series of experiments to determine how straight nocturnal dung beetles roll under the open sky with (1) polarised light from the moon available as the main orientation cue, (2) with only stars available as an orientation cue and (3) with little caps fitted over the dorsal eyes of the beetles to greatly reduce the availability of celestial cues for orientation. The beetles were released with their dung balls from the centre of a circular arena (diameter 3 m) and filmed from above. Rolling tracks were reconstructed from the films and their straightness determined over a radial distance of 120 cm. Tracks of beetles prevented from seeing a full view of the night sky become significantly longer (477 ± 75 cm) than when the beetles are allowed to view either a moonlit (133 ± 2 cm) or a starlit sky (185 ± 12 cm). This indicates that the beetles can orient to the stars as well as to the moon. To test our theory that the beetles are able to use stars for orientation, we studied the orientation performance of beetles in the Johannesburg planetarium. Since the rolling speed of beetles does not change with stimulus condition, the orientation performance of the ball-rolling beetle can be determined by timing the beetles from the moment they leave the centre of the arena until they reach its edge. In the planetarium, the beetles take the same time to reach the edge of the arena, irrespective of whether they roll under the full starry sky (4000 stars and the Milky Way) or with only the Milky Way visible. With only the 18 brightest stars projected on the planetarium dome, the beetles take significantly longer to reach the edge of the arena. This finding represents the first convincing demonstration for the use of the Milky Way for orientation in the animal kingdom, and the first demonstration of star orientation in an insect.

Visually based homing in a nocturnal spider. PS I.2

Thomas Nørgaard, Yakir Gagnon & **Eric Warrant** (Lund University)

At the darkest times of night adult males of the Dancing White Lady spider (*Leucorchestris arenicola*) walk several hundred meters across the Namib Desert sand dunes searching for females. These excursions are round trips starting and ending at a burrow and the spiders thus perform long-distance homing under very dim light conditions. They nevertheless rely heavily on vision. The combined visual fields of the spiders' eyes have a near full panoramic view of the surroundings and only the posterior median eyes are not used for navigation. Calculations of photon catches indicate that the eyes must utilize a combination of spatial and temporal pooling in order to function at night. Using only the basic spatial and temporal resolution of the eyes the spiders would only be able to see the brightest stars in the sky and not any objects in the landscape. Using spatial and temporal summation the spiders would be able to detect larger structures in the landscape. Earlier studies have shown that the spiders use landmarks when homing and indicate that they depend on extended temporal summation that allows them to see such stationary objects in sufficient detail. The exit trajectory of spiders leaving the burrow for the first time within a new area has a conspicuous sinusoidal shape. This behaviour appears analogous to the orientation flights of honey bees and could ensure that the spiders obtain a three dimensional representation of landmarks in the surroundings. If the spiders do more than one excursion during a night, consecutive exits become progressively straighter. This change in trajectory shape indicates that the spiders have the ability to perceive and remember the layout of objects in their surroundings.

Memory goes crossmodal: Visual and olfactory navigation in ants. PS I.3

Kathrin Steck, Markus Knaden & **Bill Hansson** (Max Planck Institute for Chemical Ecology, Jena)

The desert ant, *Cataglyphis fortis*, uses both visual (Wehner 2003) and olfactory (Steck et al. 2009) cues to guide its return to its nest. This natural behavior provides us with a sensitive method for examining the time course of unimodal and bimodal learning under field conditions, which has yielded striking results. Ants are relatively slow to learn the location of the nest when it is specified by just an olfactory or a visual cue. But learning is accomplished in one single trial when visual and olfactory cues are provided together as a bimodal compound. Initially, ants trained with the bimodal compound responded as accurately to visual or olfactory elements presented singly as they did to the compound, but after 15 trials the compound evoked an accurate response, but single elements did not. This dramatic change in associative strength between elements and compound depending on the degree of experience might prevent confusion arising from environmental ambiguity and thus help *Cataglyphis* to navigate within visually cryptic habitats.

Behavioral relevance of polarization sensitivity as a target detection mechanism in cephalopods and fishes. PS I.4

Vincenzo Pignatelli, Shelby Temple, Tsyr-Huei Chiou (The University of Queensland), **Nicholas Robert** (Bristol University), **Shaun Collin** (The University of Western Australia) & **Justin Marshall** (The University of Queensland).

Aquatic habitats are rich in polarized patterns that could provide valuable information about the environment to an animal with a visual system sensitive to polarization of light. Both cephalopods and fish have been shown to behaviorally respond to polarized light cues, suggesting that polarization sensitivity (PS) may play a role in improving target detection and/or navigation/orientation. However, while there is general agreement concerning the presence of PS in cephalopods and some fish species, its functional significance remains uncertain. Testing the role of PS in predator or prey detection seems an excellent paradigm with which to study the contribution of PS to the sensory assets of both groups, because such fundamental behaviors are critical to survival. We developed a novel experimental setup to deliver computer-generated, controllable, polarized stimuli to free swimming cephalopods and fish with which we tested the behavioral relevance of PS using stimuli that evoke innate responses (such as an escape response from a looming stimulus and a pursuing behavior of a small prey-like stimulus). We report consistent responses of cephalopods to looming stimuli presented in polarization and luminance contrast however, none of the fishes tested responded to either the looming or the prey-like stimuli when presented in polarization contrast.

The environmental skeleton: Tension-based locomotion in a soft-bodied climbing insect. PS I.5

Barry Trimmer, Michael Simon & **Huai-ti Lin** (Tufts University)

An outstanding problem in animal locomotion is to explain how soft animals control their movements. Most models of soft bodied locomotion assume that force transmission is mediated by a hydrostatic skeleton. Using a variety of experimental approaches on *Manduca sexta* we now show that caterpillars do not necessarily rely on hydrostatics for crawling and climbing. We have identified five novel results relating soft body mechanics to the neural control: (1) Manduca caterpillars crawl horizontally and climb vertically using the same kinematic gait. (2) In electromyographic recordings from identified muscles in freely crawling Manduca the activity of major dorsal and ventral muscles are not functionally antagonistic and there is substantial overlap in activity between longitudinal and oblique muscles in different segments. (3) The first measurements of caterpillar surface reaction forces at multiple contact points suggest a new model of caterpillar crawling in which compressive forces are carried by the substrate; locomotion is based on the sequential release of tension through controlled changes in grip. (4) Using synchrotron-sourced phase contrast X-rays, we found that the gut slides forward in mid-body segments before the body wall itself, thereby causing a shift in the center of mass of the animal during the stance phase. (5) Manduca body volume is not necessarily constant during movements and pressure is not uniform in the body or well-correlated with movements. Together these findings suggest that, in contrast with the hydrostatic systems employed by annelids and mollusks, Manduca uses an “environmental skeleton” that neither depends on controlling internal pressure nor requires antagonistic muscle activity. One advantage of this strategy is that Manduca can remain comparatively soft and conform to the substrate for stability and camouflage. This discovery is being used in the design, construction and control of a new class of terrestrial, soft material robots.

Echolocation strategy of free-ranging FM bats, Pipistrellus abramus, during target search, revealed by a microphone-array system. PS I.6

Emyo Fujioka, Naohiro Matsuta, Shizuko Hiryu, Hiroshi Riquimaroux, & **Yoshiaki Watanabe** (Doshisha University)

Aerial-feeding insectivorous bats capture hundreds of small airborne insects a night. Here, we measured sonar emissions and 3-D flight paths of wild *Pipistrellus abramus* during foraging insect preys by using a custom made microphone-array system consisted of four units. Each unit was consisted of four 1/8-inch omni-directional condenser microphones arranged in a symmetrical Y-shaped formation. Two units facing same direction were installed on one side of a riverbank and the other two units rotated opposite direction were set up on the opposite bank, facing the area where the bats have been observed. The 3-D flight paths of foraging bats were continuously tracked while the bats captured multiple insects within 10-15 squared meters and 4-9 meters high above the river surface for almost 1 minute. The flight areas got larger three-dimensionally and showed simpler circular pattern when the capture repetition rate was low (1 capture in 22 s) compared to the condition when the capture repetition rate was high (22 captures in 49 s). In the search phase, the bats emitted quasi constant-frequency (CF) pulses, and the CF duration increased as a function of flight altitude. The interpulse interval (IPI) was relatively constant at around 90-110 ms during the search phase. The search range estimated from the IPI of 90-110 ms was 5-9 meters which approximately corresponded to the flight altitude of the bats. Interestingly, *P. abramus* prolonged IPI occasionally longer than 150 ms (long IPI), which was observed frequently in the case of low capture repetition rate. The estimated search range from the long IPI was 15-24 meters which approximately corresponded to the area where the bats have been intensively observed. This finding suggests that the bats may frequently expand their search range by prolonging IPI in order to find new foraging area when the insect density is low, which might be an effective search strategy used by bats.

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Salamander tongue projection is based on target motion extrapolation.

PS II.1

Bart G. Borghuis, Anthony Leonardo (HHMI - Janelia Farm Research Campus)

For a predator, successful prey capture is a matter of life or death, and the neural circuits driving it should be robust to variations in hunting conditions. To learn how these conditions influence the behavioral strategy, we placed a freely moving salamander (*eurycea*) and a freely moving fruit fly (*drosophila*) in an arena and quantified 272 high speed video clips of tongue projections over a 5 log-unit range of light intensities, and a wide range of target positions, sizes and speeds. Capture success was 60% in scotopic light (0.005 µW/cm2), peaked at 90% in mid-photopic light (10 µW/cm2) and fell to 70% in high photopic light (200 µW/cm2). Tongue projection distance varied linearly with target distance over a 4 to 27 mm range and the projection depth error was small (1.7 ± 2.5 mm). Tongue projections were ballistic and had the same acceleration and velocity profile independent of distance. Angular error was greater for nearby flies than for distant flies. That these errors decreased with target distance suggests they are predominantly neural, rather than kinematic in origin. The salamander maintains a two-tongue width error margin independent of target distance, allowing a sufficient safety margin to ensure capture success. Many tongue projections (61%) were preceded by a rapid head rotation. We found that an extrapolation model (in which the tongue is launched to the location the fly is moving towards when the head turn begins) predicted actual projection angles two-fold more accurately than a model which projects to the location of the fly prior to the head turn. The salamander appears to estimate both position and velocity of its target in a window 100-400ms before tongue projection, and uses this information to make a precise head turn culminating in a launch of the tongue. Projection errors increased when the fly accelerated during the head turn, suggesting that the extrapolated fly position is not strongly modified by visual feedback during the head turn.

A precise population code for attractive pheromone mixtures. PS II.2

Joshua Martin, Hong Lei, Jeffrey Riffell, Eleni Constantopoulos & **John Hildebrand** (University of Arizona)

Odors that elicit and guide animal behavior typically consist of multiple volatile chemicals. Much has recently been learned about how individual odors and mixtures are encoded, especially in the primary olfactory center (olfactory bulb in mammals, antennal lobe in insects). However, little is yet known about whether animals utilize higher-order features of odor mixtures, e.g. the relative proportions of components, or how these features are encoded in the brain. We report that the natural, 2:1 ratio between the two major components of the pheromone of a moth (*Manduca sexta*) is most effective in eliciting mating behavior, and is encoded in the precision of synchronous spikes produced by a population of cells in the antennal lobe of the animal. Males were more likely to exhibit mating behaviors to synthetic pheromone mixtures mimicking the natural 2:1 ratio than mixtures with altered ratios. We quantified the information about the ratio of components in the mixture available in various features of the output neurons of the Macroglomerular Complex (MGC), an assemblage of antennal lobe glomeruli responsive to the pheromone. Reliable information was available in the precision of synchronous spikes between pairs of PNs in the MGC. Additionally, pairs of neurons were found to belong to one of two populations: one population responded with more precise synchrony to mixtures with greater proportion of one component, the other to mixtures with a greater proportion of the other component. In this way, synchrony is most precise in the whole population when the natural, 2:1 ratio is encountered. This mechanism likely contributes to the behavioral preference for the natural pheromone mixture.

A mechanical/sound sensitive centrifugal neuron in the central olfactory pathway of the heliothine moth. PS II.3

Xin-Cheng Zhao & **Bente G. Berg** (Norwegian University of Science and Technology)

The antennal lobe (AL) is the primary processing center for odor information in insects. This region contains a large number of sensory axon terminals making synapses with central neurons in characteristic glomerular structures. In addition to the two most numerous populations of AL neurons, the projection neurons and the local interneurons, a third category occur in relatively small numbers — the centrifugal neurons (CN). The CNs extend their dendrites in different areas of the nervous system and send an axonal projection into the AL. CNs are assumingly involved in modulating the afferent information flow in the AL, often via release of biogenic amines. By receiving input from various sensory channels, these feed back neurons are in general presumed to enhance or suppress odor guided behavior according to environmental factors. One extensively studied CN is the so-called VUMmx1, shown to facilitate the formation of memory in the honey bee. Thus, this octopaminergic neuron mediates information about the unconditioned taste stimulus in associative olfactory learning (Hammer, Nature 1993). In order to achieve knowledge about other modulatory networks, e.g. those connected to color and sound, it is necessary to characterize CNs physiologically and morphologically. Here we present a novel CN in the central olfactory pathway of the heliothine moth, identified by the intracellular recording and staining technique. The uni-lateral neuron which has its soma in the dorso-medial region of the PC projects into the AL where it gives off relatively solid branches with blebby terminals in all glomeruli. A second branch innervates the medial PC with many fine arborizations. We have identified the neuron in two heliothine species, Heliothis virescens, and Helicoverpa armigera. Interestingly, the neuron responded to air puffs applied to the antenna and to sound derived from valves when they open and close. The frequency spectrum of the sound includes that of an echolocating bat.

The work was supported by The Norwegian Research Council, project number 178860/V40.

Homologous interneurons have distinct functions in the generation of similar rhythmic motor patterns. PS II.4

Akira Sakurai (Georgia State University), **James M. Newcomb** (New England College) & **Paul S. Katz** (Georgia State University)

The nudibranch molluscs *Melibe leonina* and *Dendronotus iris* exhibit similar swimming behaviors consisting of rhythmic lateral body flexions. The swim central pattern generator (CPG) in *Melibe* consists of 2 bilaterally symmetric swim interneurons (Si1 and Si2) that fire bursts in alternation with their contralateral counterparts because of mutual inhibition (Thompson & Watson, JEB, 2005). Here we found that a distinct neural mechanism produces a similar motor pattern in *Dendronotus*. In *Dendronotus*, we identified putative Si1 and Si2 homologues. The putative Si2 homologues in the pedal ganglia were mutually inhibitory and fired in an alternating bursting pattern. In contrast, putative Si1 homologues in *Dendronotus* were not mutually inhibitory and were not rhythmically active during the motor pattern. We named them the Cerebral Ipsilateral Pedal (CIP-1) neurons because of their location and characteristic axon projection to the ipsilateral pedal ganglion. Unlike the *Melibe* Si1, the *Dendronotus* CIP-1 bilaterally excited other swim interneurons. Depolarization of CIP-1 initiated a swim motor pattern or accelerated an ongoing motor pattern. Hyperpolarization of CIP-1 slowed the rhythm. Thus, the *Dendronotus* CIP-1 is not a member of the swim CPG, but plays a regulatory role in rhythmic motor pattern generation. Si1 and CIP-1 differed in their electrical connectivity. In *Melibe*, synergistic bursting of Si1 and Si2 on the same side was caused by ipsilateral electrical coupling that was approximately 10 times stronger than the coupling between the contralateral counterparts. In contrast, in *Dendronotus*, CIP-1 appeared to have evenly-distributed electrical coupling with swim interneurons on both sides. Thus, although the rhythmic behaviors of *Melibe* and *Dendronotus* appear similar and the nervous systems contain homologous neurons, there are important differences in the organization of the CPGs that produce the motor patterns.

Pattern generation and coordination in cockroach locomotion. PS II.5

Einat Fuchs (Princeton Univ & Tel Aviv Univ.), **Itzhak David** (Tel Aviv Univ.), **Philip Holmes** (Princeton Univ.) & **Amir Ayali** (Tel Aviv Univ.)

The cockroach is renowned for its remarkably stable, yet rapidly adaptable, locomotion. This has been crucial for its evolutionary success. It is also a major inspiration for the development of mathematical models of multi-legged locomotion, as well as biologically-inspired robotics. Two extreme schemes have been proposed for locomotion control; in the first, central pattern generator(s) (CPGs) coordinate rhythmic limb motion. In the second, motor activity depends on sensory inputs (feedback loops). Here we utilize a combined experimental and theoretical approach to investigate the relative importance of inter-segmental afferents versus CPG interconnections for the coordination of cockroach locomotion. We simultaneously recorded the coxae levator and depressor motoneurons in the thoracic ganglia of a walking cockroach, while sensory feedback was completely blocked, or allowed only from one specific intact stepping leg. We observed a tripod-like activity gait in the absence of sensory feedback, suggesting the existence of central feed-forward control. In addition, we show transient stabilization of phase differences between the middle and hind thoracic motoneurons following individual steps of a front leg, suggesting that phasic information from a stepping leg can stabilize phase relations between the other components of the circuit activating the different legs. Data were further analyzed using stochastic models of coupled oscillators and maximum likelihood techniques to estimate underlying physiological parameters, such as uncoupled endogenous frequencies and the strength and direction of coupling. Our findings indicate that descending ipsilateral coupling is stronger than ascending, while left-right coupling in both the meso- and meta-thoracic ganglia appears to be symmetrical. A comparison with recent findings in stick-insects may indicate different inter-segmental coordination strategies in the two insects that exemplify opposite extremes of a fast-slow locomotion continuum, while sharing much of the neural and body architecture.

It's about time: How input timing is used and not used to create emergent properties in the auditory system. PS II.6

Joshua Gittelman, Na Li & **George Pollak** (University of Texas at Austin)

Frequency Modulations (FMs) are major components of bat echolocation and communication calls, and are common in communication across species, including humans. Previous work has shown that neuronal response selectivity for FM direction often predicts response selectivity to conspecific vocalizations. For over 35 years, the major hypothesis for FM directionality invokes a mechanism with an honored tradition in sensory neurobiology: the relative timing between excitation and inhibition. The timing disparity is created by the asymmetrical locations of excitatory tuning and inhibitory sidebands. Inhibitory sidebands tuned to frequencies lower than excitation creates downward selectivity. FMs sweeping downward activate excitation first; the initial excitation is unopposed by inhibition, evoking discharges. Upward FMs evoke inhibition that either leads or is coincident with the excitation, suppressing discharges. Here we evaluated FM directionality with in vivo whole-cell recordings from the inferior colliculus (IC) of awake bats. From the recordings, we derived synaptic conductance waveforms evoked by down- and upward FMs that swept identical frequency ranges. In a model, the derived conductances accurately predicted sound-evoked postsynaptic potentials (PSPs). When we tested the effects of shifting inhibition relative to excitation in the model, we found that EPSP amplitudes were remarkably insensitive to small latency shifts (often < 1 mV/ ms). Importantly, the effect of shifting inhibition depended strongly on initial differences in the latencies and shapes (temporal envelopes) of excitation and inhibition. Finally, when the PSPs peaked close to spike threshold, even small latency shifts could cause some cells to fire more strongly to a particular FM direction, and thus change its directionality. We found that timing is more than latency differences between excitation and inhibition, and that directionality depends on a complex interaction between conductance temporal envelopes, size, and spike threshold.

Symposium 7

"Sensory Neuroecology: The Sensory-Neural Bases of Natural Behavior Viewed from an Environmental Perspective"

Organizers: **John Hildebrand** and **Jeff Riffell** (University of Arizona)

Escape by inking: the neuroecology of predator avoidance by inking molluscs.

S 7.1

Charles Derby (Georgia State University)

Inking is a striking behavior of marine molluscs such as sea hares, octopus, squid, and cuttlefish. Many people assume, though with little experimental support, that inking functions as an antipredatory defense by acting as a 'smoke screen' or visual decoy. Another possibility, which has received even less consideration, is that inking functions in the chemical realm. My research group has found that sea hare ink acts on the chemosensory systems of would-be predators such as crustaceans (spiny lobsters, blue crabs), fish (sharks, sea catfish, wrasses), and sea anemones through an impressive array of mechanisms. These include 'phagomimicry', sensory disruption, and deterrence using aversive substances. Ink also functions in defense through alarm cues – sea hares show escape behavior when they detect ink from conspecifics. The chemical deterrents and alarm cues are diverse in molecular structure, numerous, and include both diet-derived and de novo synthesized molecules. Some have been co-opted from other functions, including sun screens and antimicrobials. Recent studies show that some principles demonstrated for sea hares also apply to Caribbean reef squid: ink protects squid from predatory fish through deterrent compounds and conspecific alarm signaling. Thus, using ink in both the chemical and visual realms may be a common defensive mechanism for inking animals. These modes of chemical defense contribute together with other defenses to protect inking animals from predators.

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Getting over background noise: Mechanisms underlying acoustic communication in adverse environments. S 7.2

Victoria S. Arch, Dwayne D. Simmons, Patricia M. Quiñones & **Peter M. Narins** (University of California).

Biotic and abiotic environmental noise is ubiquitous in the habitats of sonically communicating organisms and can mask vital intraspecific vocalizations. The risk of masking is contingent on the frequency and temporal overlap between the signal and the ambient noise. As a result, some species have evolved to place their calls within "silent windows" of their environment. The Southeast Asian frogs *Odorrana tormota* and *Huia cavitympanum* call adjacent to rushing montane streams and waterfalls that produce high-intensity sound spanning the human audible spectrum (*ca*. 20 Hz – 20 kHz). Both species communicate with ultrasonic frequencies (> 20 kHz), making them the first non-mammalian vertebrates shown to do so. We hypothesize that these distantly related species converged on ultrasonic communication to improve the signal-to-noise ratio of their calls amidst the broadband background noise of their habitats. We used histology, immunohistochemistry and confocal microscopy to compare the morphology of the peripheral auditory systems of *O. tormota* and *H. cavitympanum,* which have upper hearing limits of 34 and 38 kHz, respectively, with those of *Rana pipiens*, a species with an upper detection limit of *ca*. 3 kHz. Our results suggest that the ultrasonic species have converged on critical, small-scale functional modifications of the auditory periphery that subserve high-frequency detection. These data present the intriguing possibility of convergence in the communication behavior and underlying sensory physiology of frogs that have evolved in environments characterized by broadband background noise. However, we will also discuss the apparent rarity of ultrasonic communication among amphibians, which raises interesting questions regarding the ecological and physiological constraints that may prevent the widespread use of this communication strategy.

Predicting diversity in visual sensitivities, signals and behavior: an environmental approach with kelp forest fishes. S 7.3

Molly Cummings (The University of Texas)

To optimize information extraction from any given environment, sensory tuning often produces biases that influence the evolution of communication traits. For dichromatic fishes living in the optically variable California Kelp forest, species-specific differences in optical habitats promotes the divergence of detectional biases for brightness or color contrast detection. I combine species-specific optical and physiological measurements with ancestral estimates in a visual detection modelling approach to examine bi-directional biases in sensory and signalling traits across five surfperch species inhabiting the Californian kelp forest. Species-specific divergence in visual pigments correlates with changes in environment and produces different sensory biases. Divergence in male signals (color pattern reflectance and display behavior) is predicted by each species' sensory bias: signal divergence favors chromatic signal detection for species with chromatically biased visual systems, while species with luminance sensory biases have signals favoring luminance detection. This quantitative example of co-evolution of communication traits varying in a bi-directional pattern governed by the environment is one of the first demonstrations of sensory trade-offs driving signal evolution.

Sensory constraints and behavioural options in fiddler crabs. S 7.4

Jan Hemmi (The Australian National University)

Mating systems, foraging strategies and movement patterns of animals, as they are studied by behavioural ecologists, all involve information processing, both at the sensory and the cognitive level. In order to identify what behavioural options animals have and to understand the subtle selective forces that have shaped and continue to shape the behavioural ecology of animals, we require an understanding of information processing under evolution-relevant conditions. Sensory constraints limit the amount and accuracy of information animals have available when making decisions. In predator-prey interactions, for instance, the perceptual abilities and the sensory ecology of prey animals have a major influence on their behavioural options with far-reaching consequences for their lifestyle. In fiddler crabs we are able to measure the animal's behavioural responses together with the exact visual information these animals have available during predation events while the animals are operating in their natural social and physical environment. This allows us to measure the visual information fiddler crabs use to make decisions about whether and how to respond to predators. In this talk, I will explore the relationship between the fiddler crabs' eye design, the information they are able to extract from the sensory input stream and the organisation their anti-predator responses. Fiddler crabs have limited information about the size and distance of approaching objects. I will show that this simple limitation forces the crabs to respond very early and indiscriminately to approaching objects and that it dominates and shapes the entire organisation of their daily lives, including their anti-predator response strategy.

Symposium 8

"Habituation – an Evolutionary Conserved Mechanism of Sensory Information Processing?"

Organizer: **Susanne Schmid** (University of Western Ontario)

High throughput behavioral characterization of habituation in wild-type *C. elegans* and a mutant library of nervous-system-biased strains. S 8.1

Catherine Rankin, **Andrew Giles** (University of British Columbia), **Nicholas Swierczek** & **Rex Kerr** (Janelia Farm Research Campus HHMA)

Habituation is the most simple and fundamental form of learning and is measured as a decrease in response to a repeated stimulus. We assay habituation of the tap withdrawal response in C. elegans using the Multi-Worm Tracker (MWT). The MWT allows rapid characterization of tap habituation and therefore enables the screening of large numbers of mutants. Typically, we track 60-80 animals at a time on a single Petri plate seeded thinly with E. coli. We observe spontaneous behavior for 10 minutes prior to the stimulation protocol to assay the strain"s locomotion and spontaneous reversal rates. Thirty mechanical taps to the side of the test plate are then administered at a 10 second inter-stimulus interval. In wild-type worms, the tap initially elicits a reversal response that gradually habituates with repeated presentations. We have observed approximately 50,000 worms from various wild-type strains across 800 independent experiments tested across a number of months. We will present details of the behaviors we have observed and how they vary within the wild-type strains. We have also collected a nervous-system-biased mutant library (~700 strains) by cross-referencing a list of 2073 gene with predicted neural function based on domain structure (Sieburth et al. Nature:436, 2005) with the list of available strains at the Caenorhabditis Genetics Center. We have tested a majority of these strains (four replicates each) and will also discuss variation of the behaviors within and between the mutant strains. In particular, we have verified the defects in known tap habituation mutants and found new mutants that have relatively wild-type behavior on all measures save the rate of habituation of the tap withdrawal response. Thus, we expect that the mutants isolated in this screen will yield new insights into the molecular mechanism of habituation.

New insights into the cellular mechanisms of habituation in *Aplysia.* S 8.2

David L. Glanzman (Brain Research Institute, UCLA)

Despite representing perhaps the simplest form of learning, habituation remains poorly understood in term of its underlying cell biology. The gill withdrawal reflex (GWR) of the marine snail *Aplysia californica* has served as an important model system for cell biological studies of habituation. Traditionally, it has been thought that habituation of the GWR is due to depression of transmitter release at the presynaptic terminals of sensory neurons in the abdominal ganglion that mediate the reflex. However, recent work has implicated postsynaptic mechanisms in long-term habituation (LTH) of the GWR, particularly activation of AMPA-type and NMDA-type postsynaptic glutamate receptors. In addition, LTH depends on activation of protein phosphatases, including protein phosphatase 1 (PP1) and calcineurin (PP2B). Taken together, the available data suggest LTH may be mediated, in part, by homosynaptic long-term depression (LTD). But heterosynaptic mechanisms may also play an important role in LTH. Our evidence indicates that heterosynaptic inhibition mediated via a pathway involving FMRFamide-containing interneurons may contribute to LTH. Thus, habituation of the GWR in *Aplysia* appears to involve both pre- and postsynaptic mechanisms, as well as homosynaptic and heterosynaptic pathways.

Long-term habituation. Is it really a non-associative process? S 8.3

Daniel Tomsic (University of Buenos Aires. CONICET ARGENTINA)

Habituation is commonly classified as a nonassociative form of learning because it is assumed to be governed solely by the parameters of the habituating stimulus, in the absence of associations with other stimuli. Such a general assumption may be wrong. As proposed by Allan Wagner short-term habituation (STH) could be entirely nonassociative while long-term habituation (LTH) may entail associations with the contextual environment. But, because the vast majority of studies concentrated on STH, the notion that habituation is purely nonassociative is deeply embedded in the literature and in people's mind. In my talk I will summarize results from an extensive program aimed at investigating the reduction of the escape response upon repeated presentation of a visual danger stimulus in the crab *Chasmagnathus*. Presentation of the stimulus without intertrial intervals (continued training) renders only STH. High frequency stimulus presentation (massed training) results in intermediate-term (1 day) habituation (ITH), whereas low frequency stimulation (spaced training) results in long-term memory (>5 days). LTH, but not ITH, is protein synthesis and context dependent. Experiments show that in fact, LTH is determined by an association between a memory of the habituating signal stimulus (signal memory, SM) and a memory of the context (context memory, CM). In vivo intracellular recording during learning allowed the identification of neurons from the crab's brain that subserves every attribute of the SM, but they do not support the CM or the context-signal memory association (CSM). Our studies in *Chasmagnathus* illustrate that STH, ITH and LTH of the escape response (to the same visual stimulus) involve different neural substrates and mechanisms. These and further studies show that the commonly accepted definition that habituation is a simple nonassociative form of learning is misleading.

Mechanisms mediating habituation in rodents. S 8.4

Susanne Schmid (University of Western Ontario), **Peter Ruth** & **Peter Pilz** (University of Tuebingen)

Habituation to sensory stimulation provides an important sensory filter mechanism and is also considered being the most basic form of learning. Short-term habituation has often been assessed as habituation of an escape response. Studies using the gill withdrawal reflex in Aplysia or startle responses in rats have indicated a presynaptic and calcium dependent mechanism at the sensorimotor synapses of the reflex pathway as underlying mechanism. The molecular mechanism, however, has never been fully resolved. In line with these findings, we here demonstrate that the activation of a large-conductance calcium-activated potassium channel, the BK channel, mediates short-term habituation of startle responses in rats and mice using genetic and pharmacological models: Disruption of the highly conserved slo-1 gene that codes for the pore forming alpha subunit of BK channel leads to an attenuation of startle and a disruption of short-term habituation of acoustic startle responses in mice. When wild-type and knock-out mice are matched with respect to their startle amplitudes, the lack of short-term habituation persists in knock-out mice, excluding a floor effect as a cause for the lack of habituation in the BK knock-out mice. In rats, local injections of the BK channel blocker iberiotoxin into the caudal pontine reticular nucleus where the sensorimotor synapses of the startle response are located, disrupts short-term habituation without affecting baseline startle responses as compared to saline injections. We here show a total disruption of short-term habituation of an escape response in mammals without affecting the baseline response, thereby identifying an important component of the habituation mechanism. Our results confirm similar results in Drosophila and C. elegans, indicating that the BK channels activation is a common and highly conserved molecular mechanism mediating short-term habituation of startle ranging from nematodes to mammals.

Symposium 9

"Spatial orientation and object identification with sensory arrays: from neurons to robots"

Organizers: **Joachim Mogdans** & **Horst Bleckmann** (University of Bonn)

Air flow guiding a spider to prey passing by in flight. S 9.1

Friedrich Barth & **Christian Klopsch** (University of Vienna)

The successful jump of a wandering spider (*Cupiennius salei* Keys.) towards insect prey passing by in flight is a demanding behavior. Arrays of highly evolved air flow sensors (trichobothria) on the legs are used by the spider to detect the cues contained in the flow generated by a flying insect and guiding the spider to the location of its prey with the necessary temporal and spatial precision. The application of digital Particle Image Velocimetry (DPIV) allowed us to gain a highly resolved picture of the air flow close to the spider sensors and to identify the cues most likely used for prey localization. Behavioral experiments in which different components and characteristic features of the air flow were used to stimulate the spider strongly support this view.

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Active flow-sensing for spatial exploration and navigation. S 9.2

Sheryl Coombs (Bowling Green State University)

Blind cavefish (*Astyanax mexicanus*) are unable to scan their surroundings from a single vantage point by visual or other long-range sensory systems to determine the spatial configuration of their distant surroundings. Rather, they must rely on short-range senses and swim within sensory range of each landmark feature. Thus, any knowledge of the spatial relationship between two or more features must be obtained from sequential encounters. In order to sense nearby features without touching them, fish use active-flow sensing to detect the spatiotemporal perturbations caused by nearby stationary objects in their own self-generated flow fields. Given that flow signal generation and reception is coupled to the coast phase of their burst-coast swimming gait, sensory updates about their position in space with respect to their surroundings are intermittent and constrained by locomotor demands. As a consequence, spatial exploration and navigation pose special challenges for blind cavefish. Comparative studies on the swimming trajectories and fine-scale swimming kinematics of blind cavefish and their nearest sighted relative, a morph of the same species, reveal interesting similarities and differences in the sensorimotor strategies used by these two morphs when exploring novel environments. Comparisons suggest that both morphs share common strategies for regulating the temporal characteristics of burst-coast swimming kinematics, but that blind morphs differ significantly from sighted morphs in their swimming trajectories and in lateral line-enabled abilities to link swim cycles into sequences that form straight trajectories. These differences can best be understood in terms of the intermittent and short-range challenges of active flow-sensing by blind cavefish and suggest that these fish have evolved behavioral strategies for coping with these challenges.

Deciphering ambiguous electro-sensory images with arrayed structures. S 9.3

Jacob Engelmann (University of Bielefed) and **Gerhard von der Emde** (University of Bonn)

Weakly electric Mormyrid fish are endowed with a variety of arrayed sensory structures embedded in the skin of these animals and are used for navigation, orientation and even communication. Of special interest for our research is an array of two differently tuned electroreceptors used for active near-range navigation and object detection. In addition to these, Mormyrids possess an array of electroreceptors for passive electrolocation and an array of mechanoreceptors. This makes Mormyrids focal models to investigate the sensory benefits of neural processing using multiple two-dimensional sensor arrays.

Here, we present our recent advances in the quantitative analysis of object inspection abilities of mormyrid fish, showing that active electrolocation can resolve physically ambiguous stimuli and lead to precise non-visual environmental imaging. Extending on this issue, we will show data derived from both measured and modeled stimuli used in behavioral experiments to elucidate the problems associated with the reconstruction of the complex 3-dimensional electrosensory environment by use of 2-dimensional sensory arrays. Several pre-receptor and neuroanatomical mechanisms will be presented that can be seen as adaptations to cope with this problem.

Stepping from the peripheral specializations to central mechanisms, we will discuss how simple environmental cues are represented at the first central station in the brain and how orientation-specific parameters might be read out at higher stages. The benefit of several arrays in upstream sensory processing will be addressed in this context, showing that feature enhancing contrasting mechanisms related to behaviorally relevant cues can be explained by the integration of sensory input processed in parallel.

In a comparative manner, we will relate these biological findings to challenges arising when it comes to navigation and object identification by autonomous underwater vehicles. We will present preliminary data on algorithms which can be used for electro-orientation in a robotic approach.

Patterns of input across the rat vibrissal array during object exploration.

S 9.4

Mitra J. Z. Hartmann (Northwestern University)

Rats use rhythmic movements of their mystacial vibrissae (whiskers) to tactually extract object features, including size, shape, and texture. Our laboratory uses the rat vibrissal system as a model to understand sensorimotor integration in the early stages of the nervous system. This talk will describe three advances we have recently made towards quantifying the patterns of mechanosensory input across the vibrissal array during object exploration.

First, we have developed methods to visualize vibrissae-object contact patterns as the rat explores an object. Results show that rats use a non-uniform sampling strategy characterized by periodic fixations at distinct spatial locations, with spatio-temporal jitter around the fixation points. The function of the temporal jitter may be to increase the sensitivity of the nervous system to incoming sensory data.

Second, we have quantified the morphology of the rat vibrissal array and begun to examine its influence on the vibrissae-object contact patterns generated during exploration of an object. Given only a vibrissa's identity (row and column location within the array), we have developed an equation that establishes the vibrissa's two-dimensional (2D) shape as well as its three-dimensional (3D) position and orientation. We use the morphological model to demonstrate that differences in whisker contact times with a surface can be used to discriminate surface curvature.

Third, we are developing a simulation environment to enable full dynamical simulations of vibrissal-object contact. The simulations aim to integrate realistic vibrissal dynamics with behaviorally-measured head and vibrissal kinematics to model the rat's sampling strategies for various objects in the environment. Ultimately, the simulation system will be used to predict the contact patterns in terms of forces and moments at each vibrissa base for a given exploratory sequence.

Heiligenberg Lecture

Ed Kravitz (Harvard Medical School)

"How does behavior happen? A work in progress." SL1

Male and female fruit flies compete for resources in same sex pairings. As in most species, these agonistic interactions (fights) are divided up into brief meetings (encounters) during which a variety of behavioral patterns are observed. Some of the patterns observed are the same in male and female flies: others are male (e.g., lunge, boxing, tussling) or female (e.g., shove, head butt) specific. The patterns are stereotypical, transitioning from one to the other with a certain statistical likelihood; are easily recognizable by observers; are behaviorally meaningful to combatants; yet have the interesting property that they differ in detail every time a particular pattern is used by an animal. A fundamental question is how do patterns of this sort get established in brains: or in the present case, how do male patterns get established in male brains and female patterns in female brains? The *fruitless* gene of the sex determination hierarchy of genes in *Drosophila* plays an essential role in this process. Issues to be addressed in this presentation are: do wiring differences between male and female brains account for the expression of different behavioral patterns by the two sexes; or, is the wiring similar in male and female brains but the patterns are activated differentially; or, does some combination of the two mechanisms account for the differential expression of behavioral patterns. Studies from fruit flies and lobsters will be presented along with a simplified schematic that links together neurohormones, sensory input and central pattern generators in preliminary efforts to address these complex issues. (supported by NIGMS and NSF)

AUGUST 5

Plenary Lecture 4

Cynthia Moss (University of Maryland, USA)

"Active listening in a complex environment." PL 4

Bats echolocating in a natural setting face the formidable task of sorting signals from multiple auditory objects, echoes from obstacles, prey and the calls of conspecifics. A bat's success negotiating a complex environment depends on sonar signal processing, along with flight path control and adaptive vocal-motor behaviors, which interface with 3-D spatial perception, attention and memory. Adaptive call production can shed light on the fundamental processes that underlie perception by echolocation, and indeed, a bat's active sonar behavior allows us to listen in on the signals that an animal uses to perform a variety of auditory tasks. This talk will review findings from studies of free-flying big brown bats, *Eptesicus fuscus*, engaged in target discrimination, obstacle avoidance and prey capture tasks. Data will be presented from conditions in which a bat flies alone and in which it flies with conspecifics. The data demonstrate that big brown bats 1) Construct a 3-D representation of the environment from sonar echoes, 2) Adaptively control the direction and time-frequency structure of sonar vocalizations in response to 3-D spatial information, 3) Sequentially inspect closely spaced sonar objects, 4) Invoke the use of spatial memory and acoustic landmarks, presumably to reduce information processing load, and 5) Engage in passive listening to the sonar signals of other bats. The magnitude of vocal adjustments, reliance on spatial memory, and prevalence of passive listening depend upon a bat's familiarity with its environment, task demands, and in situations with conspecifics, baseline similarity between its sonar calls and those of other bats in the vicinity. We propose that active adjustments in sonar signal design play directly into a bat's perception of complex and dynamic acoustic scenes.

Plenary Lecture 5

Astrid Prinz (Emory University, USA)

"Mechanisms of neuron and network robustness." PL5

Neuronal oscillators, especially the central pattern generator circuits that control rhythmic behaviors such as breathing, need to function reliably throughout life despite ongoing turnover of their molecular components and other perturbations. How is this stability achieved? I will discuss recent results that show how parameter non-uniqueness, membrane conductance co-regulation, and activity-dependent homeostatic regulation through negative feedback loops act together to ensure reliable neuronal network function. My presentation will highlight how fruitful interactions between electrophysiology experiments and numerical modeling can advance our understanding of complex system dynamics.

Symposium 10

"Environmental Cues Guiding Behavior: Influence of ethanol on behavior"

Organizer: **Henrike Scholz** (University of Köln)

Variation in ethanol resistance among natural populations of *Drosophila melanogaster*: genetic basis and adaptive significance. S 10.1

James D. Fry (University of Rochester)

Ethanol occurs naturally in the decaying fruit in which many Drosophila species breed. In the model species *D. melanogaster*, resistance to ethanol varies clinally, with temperate populations having high ethanol resistance compared to ancestral tropical populations (and indeed most other Drosophilids). My laboratory is investigating the genetic basis and adaptive significance of this variation.

Neuronal bases of ethanol preference in *Drosophila melanogaster*. S 10.2

Andrea Schneider, Maite Ogueta, Osman Cibik, Rouven Eltrop & Henrike Scholz (University of Köln)

Preference guides an animal to a food source or mating partner. The smell of ethanol guides insects to a fermenting fruit or leave. Adult *Drosophila melanogaster* flies prefer food sources that contain up to five percent ethanol over a food source without ethanol or with higher concentrations. In the present study we investigate whether preference, sensitivity and tolerance of fruit flies to ethanol is related to alcohol metabolism, i.e. activity of Alcohol dehydroxygenase (Adh). The data strongly indicate a linkage between ethanol-induced behavior and ethanol metabolism in fruit flies: Adh deficiency resulted in reduced preference to low ethanol concentrations and reduced aversion to high ones, despite recovery from ethanol being strongly impaired. We provide evidence that the preference is mediated by an olfactory signal. Furthermore we analyzed the neuronal basis of ethanol preference and identified that the expression of Tyramine-beta-hydroxylase- the rate limiting enzyme of the Octopamine synthesis- is required for the regulation of ethanol preference.

Ethanol ingestion impairs appetitive olfactory learning and odor discrimination in the honey bee. S 10.3

Julie A Mustard (Arizona State University)

In invertebrates, the effects of ethanol on locomotion have been well characterized; however, less is known about how ethanol influences learning and memory. Understanding how ethanol affects learning is of particular interest because addiction may be a form of learning gone awry. Unlike many animals, honey bees (*Apis mellifera*) willingly consume solutions of sucrose containing up to 50% ethanol and they do not find the taste of ethanol to be aversive. In honey bees ethanol affects learning in a time and dose dependent manner. Ethanol ingested prior to conditioning causes a reduction in the ability of honey bees to form an association between an odor and a sucrose reward during associative olfactory conditioning. When fed after acquisition, ethanol doses that affected acquisition did not disrupt consolidation and/or recall, whereas lower doses of ethanol actually enhanced recall of an odor-sucrose association. Current work includes the investigation of the neural basis of these opposing effects of ethanol on learning and memory.

Dissecting acute ethanol tolerance in *Caenorhabditis elegans* S10.4

Jill C. Bettinger and Andrew G. Davies (Virginia Commonweath University)

There is wide natural variation in the acute ethanol response in humans, and such variation is strongly correlated with the lifetime propensity to abuse alcohol; people with a low initial level of response to ethanol are more likely to become alcoholic (Schuckit, 2002). Level of response has a significant genetic component. It is complex, and consists of at least two components, initial sensitivity to alcohol, and the rate at which acute functional tolerance (AFT) develops. AFT reflects rapid adaptation of the nervous system to the intoxicating effects of the drug, and can be observed as a decrease in behavioral impairment at the same tissue ethanol concentration during a single drug exposure. We study AFT using the *C. elegans* model because of the powerful genetic tools available, and because the worm nervous system is an excellent model for studying the genetics of behavior. *C. elegans* develop acute tolerance; in response to exposure to a constant ethanol concentration, worms undergo an acute adaptation (acute tolerance) to the presence of ethanol that does not reflect a decrease in internal ethanol concentration. Previously, we showed that the worm Neuropeptide Y receptor homolog, NPR-1, antagonizes the development of acute tolerance in worms (Davies *et al.*, 2004).

To explore the molecular mechanisms of AFT, we have performed a genetic screen for animals that are unable to develop acute tolerance, and have recovered 28 mutants. We have identified the mutated genes in two of these mutants, and found that they encode two interacting members of a transcription factor complex. This suggests that these genes regulate the expression of genes that are involved in making neurons competent to develop tolerance to ethanol. We are currently characterizing the roles of transcriptional regulation in neuronal function and ethanol response in *C. elegans*, and we are pursuing molecular characterization of the other genes identified in this screen.

Symposium 11

"Living in a Flying Crowd"

Organizer: **Peter Simmons** (Newcastle University)

Mechanisms and consequences of behavioural gregarization in desert Locusts.

S 11.1

Stephen Rogers (University of Cambridge)

Desert Locusts (*Schistocerca gregaria*) are notorious for aggregating into vast migrating swarms, but they can exist in a form that not only does not swarm, but actively avoids other locusts. The radical, but fully reversible, transformation between the solitarious and the swarming gregarious phases encompasses extensive changes in behaviour, physiology, and morphology. This makes locusts a powerful model with which to analyse the mechanisms and functional consequences of neuronal plasticity. Phase change occurs over many different time scales from hours to generations, since phase characteristics can be inherited via an epigenetic mechanism. Our recent work has concentrated on the process of behavioural gregarization that occurs when solitarious locusts change from avoiding each other towards mutual attraction. This is the first step in the process of swarm formation. Solitarious locusts acquire most of the behavioural characteristics of the gregarious phase after receiving just 2-4 hours of appropriate stimuli. A broad sweep analysis of changes in neurochemistry during the entire process of phase change revealed that serotonin increases dramatically when gregarization is established. We have now shown that serotonin is sufficient and necessary to cause behavioural gregarization. Using a combination of classic pharmacological intervention and RNA interference we have demonstrated that protein kinase A (PKA), an evolutionarily conserved mediator of neuronal plasticity, is a primary target of the initial serotonin-mediated stage of gregarization. We are also interested specifically in how altered neuronal function underlies changes in behaviour between solitarious and gregarious locusts. A large visual interneurone, the descending contralateral motion detector (DCMD), is used as an example to show how altered sensory environments and behavioural requirements are underpinned by changes in neuronal circuits.

Predator avoidance by locusts in a swarm. S 11.2

Claire Rind (Newcastle University) & **Roger Santer** (Department of Life Sciences, Limerick)

Locusts have evolved a high level of sensitivity to looming objects, probably as an adaptation to predation and a swarming life style. This sensitivity is mediated by two large identified neurons: the lobula giant movement detector (LGMD), and its postsynaptic target, the descending contralateral movement detector (DCMD). These neurons respond most strongly to rapidly approaching objects. The DCMD excites flight motoneurones and can trigger predator avoidance during flight: in response to a looming stimulus a tethered, flying locust will perform a 'gliding dive', in which it ceases to beat its wings for one or more wingbeat cycles; the wings are held elevated in stereotyped gliding posture. These gliding dives are evasive maneuvers and only occur in response to approaching objects when a DCMD produces consecutive spikes at a frequency of at least 150 Hz during the elevation phase of a wingbeat cycle (Santer R.D et al J Neurophysiol 95 3391-3400, 2006). When the locust is in an aroused state visual processing by the LGMD is altered in particular the prolonged periods of high frequency that trigger evasive glides are enhanced. Applying a brief mechanical stimulation to the hind leg or flight itself causes the DCMD response to recover from a previously habituated state, so that it is significantly more likely to generate the maintained spike frequencies capable of evoking gliding dives even with extremely short intervals (1.8 s) between approaches. In tethered flying locusts 41% responded with a glide to 6 images of approaching objects, separated by 1.8s. Octopamine may be responsible for the effect because injecting the neuronal octopamine receptor antagonist, epinastine, into the haemolymph reduced the number of glides from 41% to 12%. Consistent with this role ultrastructural studies show putative octopaminergic terminals, identified by their dense core granules, in close proximity to the input dendrites of the LGMD in the lobula.

Scale free correlations in starling flocks: A tool to enhance anti-predatory response? S 11.3

Andrea Cavagna (ISC-CNR)

From bird flocks to fish schools, animal groups often seem to react to environmental perturbations as if of one mind. Most studies in collective animal behaviour have aimed to understand how a globally ordered state may emerge from simple behavioural rules. Less effort has been devoted to understanding the origin of collective response, namely the way the group as a whole reacts to its environment. Yet collective response is the adaptive key to survivor, especially when strong predatory pressure is present. Here we argue that collective response in animal groups is achieved through scale-free behavioural correlations. By reconstructing the three-dimensional position and velocity of individual birds in large flocks of starlings, we measured to what extent the velocity fluctuations of different birds are correlated to each other. We found that the range of such spatial correlation does not have a constant value, but it scales with the linear size of the flock. This result indicates that behavioural correlations are scale-free: the change in the behavioural state of one animal affects and is affected by that of all other animals in the group, no matter how large the group is. Scale-free correlations extend maximally the effective perception range of the individuals, thus compensating for the short-range nature of the direct inter-individual interaction and enhancing global response to perturbations. Our results suggest that flocks behave as critical systems, poised to respond maximally to environmental perturbations.

Recognition versus suppression for discriminating against interfering echoes in bat biosonar. S 11.4

Mary Bates & **James Simmons** (Brown University)

Recent experiments have demonstrated that flying big brown bats (*Eptesicus fuscus*) make small changes in the frequency of their biosonar sounds in acoustically cluttered environments and when in close proximity to other echolocating bats. In flights with two bats, the principal change was shifting of harmonic frequencies very slightly (<5 kHz) away from each other and from frequencies used when flying alone. Changes in ending frequency have been associated with a jamming avoidance response in big brown bats and could indicate attempts to avoid interference while flying with conspecifics in an enclosed space. Big brown bats apply their time-frequency sensitivity for changes in echo spectrograms to suppress interference from dense, distributed clutter and also from echolocation sounds of conspecifics. In effect, spectrograms of multiple-harmonic FM sounds are treated as acoustic ¡§fingerprints¡¨ for matching echoes to broadcasts. New delay discrimination experiments with echoes having harmonics separated electronically and then offset in time, or with frequencies removed, reveal 1-3 ƒÝs sensitivities for disruption of harmonic alignment with collateral reduction of clutter interference. Bats thus may use small pulse-echo spectrogram mismatches to achieve high delay acuity as much for echo recognition and clutter rejection as for perception of delay as such.

Symposium 12

"Visual-auditory integration: converging evidence from animal physiology and behavior."

Organizer: **Yoram Gutfreund** (Technion University)

A cortical-midbrain dialogue during early life defines how the brain will integrate information from different senses. S 12.1

Barry E. Stein (Wake Forest University)

A remarkable feature of the brain is its ability to synthesize information from different senses to create a singular percept of the external world. This process of "multisensory integration" takes place at many loci, but has been best studied in the cat superior colliculus (SC), a midbrain structure involved in orientation/localization behavior. However, its underlying principles appear to be species-independent. SC neurons integrate information from various combinations of visual, auditory, and tactile inputs; a process that dramatically alters their responses and the behaviors that depend on them. Cross-modal stimuli appearing to be derived from the same event have preferential access to SC neurons, and can produce striking enhancements in activity. Those likely to be associated with different events are either not integrated or degrade responses. Contrary to some developmental theories, these abilities are not present in the newborn's brain, and their characteristics are not pre-specified. Rather, the acquisition of multisensory integration capability, and the crafting of its operational principles are postnatal processes depending heavily on at least 2 interrelated factors: a dialogue between SC neurons and their descending inputs from association cortex, and experience with the statistics of cross-modal events. The latter appear to be coded in the former and shared with SC neurons via the cortico-SC pathway. These factors help develop the neural circuitry underlying multisensory integration, and adapt its operational principles to the environment in which it will be used. They may also make it possible to modify these principles later in life if environmental circumstances change.

Supported by grants EY016716 and NS36916.

Primate vocal communication through coupled oscillations. S 12.2

Asif A. Ghazanfar (Princeton University)

When two animals communicate, how much work is each brain actually doing? How much of this work is facilitated by the structure of the communication signal itself? Our work shows that not only are the visual and auditory components of human speech tightly locked (obviating the need for the brain to actively bind such information), but the statistical regularities in both modalities are also optimized to interact with rhythms in the brain. In other words, it seems that the structure of speech exploits the structure on-going brain activity, with communication emerging as this interaction unfolds in time. Similar reciprocal coupling between signal structure and on-going brain rhythms is also seen in monkey vocal communication, and the differences between this process and human speech suggest the possibility that speech may have evolved without radical changes to key brain structures or the development of new ones.

Mechanisms of visual auditory integration in the barn owl's brain. S 12.3

Yoram Gutfreund (The Technion, Haifa)

Barn owls (*Tyto alba*) have evolved precise and sensitive auditory and visual systems to detect small prey in acoustically noisy and dimly lit environments. This specialization of the barn owl provides an excellent model system to study visual and auditory integration for target selection.

In recent years, my laboratory concentrated on studying visual-auditory integration in the barn owl's brain. Our efforts led to the discovery of two previously unknown populations of multisensory neurons; in the thalamus, nucleus Rotundus and in the forebrain, entopallium (E). These populations add to the well known multisensory neurons that exist in the midbrain (in the optic tectum). In birds, the E is the major thalamo-recipient forebrain structure of the tectofugal pathway. We have characterized visual, auditory and bimodal extra-cellular responses in the E. Experiments were conducted in lightly anaesthetized barn owls. Only a small subset of neurons responded significantly to auditory stimuli when it was presented alone. However, in almost all recording sites the visual responses were modulated by auditory signals. An auditory event which was congruent in space and time with the visual stimulus enhanced significantly the response. Interestingly, such modulation of visual responses depended dramatically on the inter-stimulus-intervals. Responses to bimodal rare events were more enhanced compared to common events. This history-dependent effect was due to strong adaptation of the neural response in the E. Thus, neural adaptation shaped not only the response magnitude but also the bimodal enhancement. I propose that this feature of the neurons in the E is in agreement with the hypothesis that the tectofugal pathway is involved in the process of detecting and orienting to the most salient event, independent of its modality.

Charley McCarthy wasn't human, but he was close enough: Cross-modal integration in a New World frog. S 12.4

Peter Narins (University of California at Los Angeles)

In the dart-poison frog *Allobates femoralis*, physical aggression against conspecific intruders is evoked only if a dynamic visual cue (vocal sac movement) is coupled with an auditory cue (advertisement call). Using an electromechanical model, we were able to experimentally uncouple these cues in time and space to test the role of bimodal integration in initiating aggression in an amphibian. Our results demonstrate that temporal and spatial integration may be reliably estimated in a freely behaving animal in its natural habitat and that we can use aggressive behavior in this species as an index of cross-modal integration in the field.

Plenary Lecture 6

Eric Warrant (Lund University)

"The ecology of vision in darkness: Vision and visual orientation in nocturnal insects." PL6

Nocturnal insects have recently revealed themselves to have formidable visual abilities despite the difficulties and limitations imposed by living in very dim light. Nocturnal hawkmoths have colour vision, nocturnal dung beetles can orientate using the dim polarisation pattern of the moon, and nocturnal bees can learn landmarks around their nest in order to find it again after returning from a long foraging trip. Much of the nocturnal visual performance of hawkmoths and dung beetles can be attributed to the superior light-gathering capacity of their superposition eyes. All bees, in contrast, have insensitive apposition eyes. Remarkably, some species are nocturnal. However, compared to those of their diurnal relatives, the apposition eyes of nocturnal species have evolved specialisations – in particular huge rhabdoms and wide facets – that endow them with about 30 times greater optical sensitivity to light. Moreover, they also have very slow photoreceptors that improve the reliability of vision at lower temporal frequencies. However, theoretical calculations, and white-noise measurements of the information capacity of their photoreceptors, both show that despite these sensitivity improvements, the photoreceptors are incapable of providing enough information at nocturnal light levels to maintain the full ommatidial resolution of the eye. We therefore believe that nocturnal insects – especially those with apposition eyes – employ spatial and temporal neural summation strategies to permit reliable vision at night. In support of this hypothesis, we have found that the lamina monopolar cells (LMCs) of nocturnal bees branch to a large number of neighbouring cartridges, and may thus provide the necessary neural substrate for spatially summing signals from overlying groups of ommatidia.

AUGUST 6

Plenary Lecture 7

Allison R. Mercer (University of Otago)

"It ain't easy being Queen: tales from the honey bee colonies" PL7

Queen mandibular pheromone (QMP) has profound effects on dopamine signalling in the brain of young worker honey bees. This complex mixture of chemicals lowers brain dopamine levels, selectively alters the levels of dopamine receptor gene expression and modifies the responses of brain tissues to this amine. At a behavioural level these changes appear to have three major consequences: aversive learning in young worker bees is blocked, their activity levels are suppressed and they are more likely to show attraction to the queen. One of the major components of QMP, homovanillyl alcohol (HVA) bears a striking structural resemblance to the biogenic amine dopamine and has recently been found to activate the honey bee D2-like dopamine receptor, *Am*DOP3. Analysis of HVA's effects is providing valuable insights into dopamine's functions in the brain while highlighting the complexity of neural circuits that guide the behaviour of this fascinating insect.

Plenary Lecture 8

Hans Hofmann (University of Texas)

"Genes, hormones and behavior: An integrative approach towards social decision making and its evolution." PL 8

We respond to the actions of others by making decisions and executing actions. How do we make these decisions, and why? Research in my laboratory aims to elucidate the kinds of decisions that prove to be most consequential in our lives: social status, mate selection and escaping from danger. To this end, we use African cichlid fishes – famous for their amazing diversity, social complexity and ease of experimentation – as our model system of choice. We have generated numerous genomic resources for cichlids and examined many of the molecular and cellular processes in the brain that are associated with social status. I will discuss how certain neuroendocrine pathways and gene expression modules are involved in establishing social dominance in males; how social status modulates startle escape performance through a reticular-spinal circuit; and how a sex hormone is necessary and sufficient to activate context-appropriate mate choice decisions. Finally, I will propose an evolutionarily conserved social decision making network as a framework for bridging the molecular and behavioral levels of behavior and its evolution.

The responses of target-selective descending neurons in the dragonfly to 3-dimensional object movements outdoors under blue sky. PS IIIa.1

Robert Olberg (Union College), **Elliot Imler** (Janelia Farm Research Campus), **Stephanie Seeman** (University of Texas), **David Shulman** (Harvard Medical School) & **Andrea Worthington** (Siena College).

Dragonflies make their living by foraging on flying insects. Eight pairs of identified neurons are implicated in controlling the dragonfly's flight path as it intercepts its flying prey. These target-selective descending neurons (TSDNs) descend from the brain of the dragonfly to the thoracic ganglia. They show directionally selective responses to small objects moving relative to the dragonfly. Their receptive fields are located in the dorso-frontal quadrant of the visual field, the region that views prey during the foraging flight. When stimulated intracellularly with high-frequency, depolarizing current pulses, each of these neurons evokes small adjustments in wing position and attitude. To understand the behavior of the TSDNs under more natural environmental conditions, we studied their responses outdoors, under blue sky, to the movement of opaque white beads of three sizes (2, 4, and 8 mm) around the immobilized dragonfly. The bead movements were videotaped (100 frames/s) for 3-dimensional reconstruction of their paths. The extracellularly recorded TSDN spikes were sorted and correlated with bead positions and velocities. We recorded from dragonflies of two genera: *Aeshna* (which forages from continuous flight) and *Pachydiplax* (which takes off to forage from a perch). The outdoor recordings revealed several new properties of the neurons and their receptive fields, three of which are presented here. (1) Receptive fields are not identical between genera, a result that may be related to their markedly different foraging strategies. (2) Three-dimensional receptive field reconstruction showed that size selectivity varies with object distance. (3) The higher light levels and ambient temperatures outdoors resulted in TSDN spike rates of 900 Hz or greater, much higher than have ever been observed in laboratory experiments.

Making an escape: Coordinating behavioral modules for flight initiation in Drosophila.

PS IIIa.2

Gwyneth Card (Janelia Farm Research Campus)

How does an animal select and coordinate a series of actions? We have identified a sequence of behaviors that the fruit fly, *Drosophila melanogaster*, performs in response to a rapidly approaching object or looming visual pattern. These behaviors enable a fly to control the direction of an escape jump that launches it into flight, away from a perceived threat. To investigate whether the suite of behaviors directing escape is a stereotyped sequence or is more flexible, I used high-speed videography to capture the responses of individual fruit flies to computer-generated looming disk stimuli presented on a high refresh rate CRT monitor. I adjusted parameters of the looming virtual disk, such as approach speed, approach duration, or contrast, to make the stimulus more or less salient. I found that flies still perform a subset of the previously observed escape behaviors, regardless of whether or not they take off in response to the stimulus. This indicates that the escape behavioral sequence is not a fixed action pattern.
Furthermore, I found that the escape sequence can be divided into separable behavioral modules whose timing and probability of occurrence are differentially affected by changing stimulus parameters. The fly also uses some of these modules when initiating flight "voluntarily" without direct external stimulus. Thus, by breaking up the takeoff sequence into individual action modules, the fly is better able to tailor its takeoff performance to its current circumstance.

The nematode touch response facilitates escape from predacious fungi.

PS IIIa.3

Mark Alkema, Sean Maguire & **Chris Clark** (Umass Medical School)

Gentle touch to anterior half of the body of free-living nematodes induces an escape response in which the animal reverses and suppresses exploratory head movements. We have analyzed the neural circuit of the escape response of the nematode *C. elegans* and found that the trace amine, tyramine, plays a crucial role in the escape response. Tyramine coordinates the backing response and the suppression of head movements through the activation of the tyramine-gated chloride channel, LGC-55. In the soil the main predators of nematodes are predacious fungi, which catch and devour nematodes using hyphal trapping devices. The most sophisticated nematophagous fungi form ring shaped traps that swell rapidly when stimulated by the touch of a nematode. We hypothesized that the touch-induced suppression of head movements could allow the animal to smoothly retreat from the constricting ring and evade capture. We therefore examined predator-prey relations between the fungus *Monacrosporium doedycoides* and *C. elegans*. Early larval stages of *C. elegans* can pass through the fungal rings and were caught most efficiently whereas adults are too large to crawl through the traps and were caught less frequently. There is a delay of approximately 5 seconds between the initial entry into the ring and trap closure, which allows the animal to withdraw from the trap before being caught. Wild-type animals managed to escape from a trap in 80% of the encounters, whereas touch-insensitive mec-4 mutants only managed to escape in 44% of the encounters. Tyramine deficient (*tdc-1*) or *lgc-55* mutants, that sense touch but fail to suppress head movements, also escaped less efficiently from traps (50-60%, respectively). In competition experiments with mixed populations of wild-type and *lgc-55* mutant animals, we found that the lgc-55 mutant animals were caught significantly more frequently. We therefore conclude that the suppression of head oscillations is an ecologically relevant behavior that allows the animal to smoothly retract from a deadly fungal noose fast enough to evade capture.

Crayfish select escape strategies based on external conditions and internal states.

PS IIIa.4

Jens Herberholz, Mary Phillips, Kenneth Sichler & **Vanessa Medley** (University of Maryland)

The behavioral and neural responses of juvenile crayfish to visual danger stimuli can be measured non-invasively using a combination of standard videography with recordings of electrical field potentials. When juvenile crayfish approach a food odor release point in an aquatic tank, they respond to sudden threat signals (shadows) with discrete behavioral outputs: freezing or tail-flipping. Tail-flips are mediated by activation of the medial giant interneurons and propel the animals away from the food source. Crayfish assess different behavioral options and make decisions based on the features of the threat stimulus, the value of the expected resource, and their current internal state. If the value of the expected food reward is high, tail-flipping is suppressed in favor of freezing. High resource value is less effective in suppressing tail-flips, however, when paired with a strong predator signal. Freezing also dominates in hungry animals whereas satiated animals produce mostly tail-flips. In addition, crayfish taken from communal tanks, where competition over food is high, freeze more and tail-flip less than isolated animals. This suggests that nutritional states and recent social experiences affect decision-making neural circuits; intrinsic needs and reward value are balanced against predation risk to produce the most desirable behavioral choice. Since tail-flipping is controlled by accessible neural circuitry, we have now begun to investigate the neural processes underlying value-based escape behavior in crayfish.

Supported by National Science Foundation grant IOS-0919845 (JH).

Number-based visual generalisation in the honeybee. PS IIIb.1

Mario Pahl (University of Würzburg and ARC Centre of Excellence in Vision Science, ANU), **Hans J Gross** (University of Würzburg), **Aung Si, Hong Zhu** (The Australian National University), **Jürgen Tautz** (University of Würzburg) & **Shaowu Zhang** (ARC Centre of Excellence in Vision Science, ANU)

Although the numerical abilities of many vertebrate species have been investigated in the scientific literature, there are few convincing accounts of invertebrate numerical competence. Honeybees, Apis mellifera, by virtue of their other impressive cognitive feats, are a prime candidate for investigations of this nature. We therefore used the well-established delayed match-to-sample paradigm, to test the limits of honeybees" ability to match two visual patterns solely on the basis of the shared number of elements in the two patterns. Using a y-maze, we found that bees can not only differentiate between patterns containing two and three elements, but can also use this prior knowledge to differentiate three from four, without any additional training. However, bees trained on the two versus three task could not distinguish between higher numbers, such as four versus five, four versus six, or five versus six. Control experiments confirmed that the bees were not using cues such as the colour of the exact configuration of the visual elements, the combined area or edge length of the elements, or illusory contours formed by the elements. To our knowledge, this is the first report of number-based visual generalisation by an invertebrate.

Visual place learning in Drosophila.

PS IIIb.2

Tyler Ofstad (Janelia Farm Research Campus/UC San Diego), **Charles Zuker** (Columbia College of Physicians and Surgeons) & **Michael Reiser** (Janelia Farm Research Campus)

Many insects use visual landmarks to precisely locate their nest, prey, or foraging area. While this behavior has been extensively studied, an understanding of the neural basis for insect place memory has been hampered by the lack of reliable tools to probe invertebrate brains. Recent advances in Drosophila molecular genetics make Drosophila melanogaster well suited for investigating the neural correlates of behavior. While the Drosophila genetic toolkit makes fruit flies an appealing organism to work with, the extent to which flies use vision to navigate and remember specific locations has been unclear. In our current studies, we show that flies do, in fact, show a robust ability to learn spatial locations. To test Drosophila for place learning, we have designed a visual place learning assay inspired by the Morris Water Maze. Rather than use water, we use heat as the aversive stimulus. To precisely control the thermal environment, we developed a thermoelectric module (TEM) array composed of 64 individually addressable 1-inch2 peltier tiles arranged in an 8x8 grid. This array forms the floor of our test arena and a LED display is positioned around the circumference to deliver visual panoramas. To test place learning in flies, we set 63 of the tiles to an aversive warm temperature and set a single tile to a preferred cool temperature. Over successive trials, flies learn to rapidly and reliably locate this cool spot using peripheral visual cues presented on the LED display. Importantly, this improvement is critically dependent on the visual panorama containing spatially distinct visual patterns. Using this assay and Drosophila molecular-genetic tools, we have identified subsets of central complex neurons required for successful place learning.

Evolution of neural networks regulating social behavior. PS IVa.1

Lauren Munchrath & **Hans Hofmann** (University of Texas at Austin)

Little is known about the evolution of the neurocircuitry that mediates important behavioral decisions, such as mate choice and social dominance behaviors. Two (partially overlapping) neural networks that seem to regulate social decision-making are the midbrain dopaminergic reward system and Newman's social behavior network. We propose that brain nuclei in these circuits are part of a social decision-making network that provides a powerful framework for understanding the evolution of social behavior and its underlying neural circuitry. We have compared neurochemical profiles of brain regions that compose the dopaminergic reward system and social behavior network across teleosts, amphibians, reptiles, birds and mammals. In this comparative analysis, we have evaluated eight neuroendocrine (sex steroid hormone receptors, neuropeptides, and neuropeptide receptors) and dopaminergic genes (tyrosine hydroxylase and dopamine D1 receptor) across the 13 brain regions encompassing this large neural network. We find a surprising degree of conservation across vertebrate classes, indicating that there is an evolutionary conserved core-SDM network already present in early vertebrates. We have found the neurochemistry of some brain regions is variable across vertebrates, but other regions are highly conserved. Throughout the whole network, we have found that where there is variation, mammals and fish are most similar given these gene expression profiles. We find that variation across the major vertebrate classes occurs with the distribution of "upstream" signaling elements, the neuropeptide- and dopamine-producing cells, whereas the distribution of "downsteam" elements, such as receptors, are very conserved. We speculate that sensory integration can vary in a species-specific manner, whereas the socially mediated decision making mechanisms have been in place for >500 million years of vertebrate evolution. Thus, the SDM network likely arose from an evolutionarily ancient core network of brain nuclei.

Neuro-economics in chicks: competitive foraging socially increases work investment and chronically enhances impulsive choices in domestic chicks.

PS IVa.2

Yukiko Ogura & **Hidetoshi Amita** (Hokkaido University)

Animals (including humans) often choose an immediate reward even when a delayed alternative option yields a larger gain. Since this behavioral trait clearly characterizes impulsiveness measured in terms of the temporal discounting, relevant neural and developmental factors have so far been intensively studied (Cardinal 2006, Kalenscher & Pennarts 2008, Matsushima et al. 2008, Trip & Wickens 2008). It has been suggested that the discounting occurs because when there is a longer delay, there is a higher risk being interrupted and consequently losing the reward, i.e., □gcollection risk hypothesis□h (McNamara & Houston 1987, Benson & Stephens 1996, Sozou 1998). Although this hypothesis is intuitively simple and plausible, the functional link between competition and impulsiveness has not been empirically supported. Here, in a series of behavioral experiments, we examined social factors involved in the control of impulsiveness and work investment in domestic chicks. In binary choices between a large / long-delay option (LL) and a small / short-delay alternative (SS), chicks that had been competitively trained in group of 3 individuals showed fewer choices of LL than non-competitive controls, even when tested 2 days after the end of training. Such a chronic effect occurred when chicks were separated through a transparent window, indicating that the actual loss of gain nor the delay-proportionate interruption was not involved. Rather, perceived competition was sufficient in the enhanced impulsiveness. The social competition also acutely increased the work investment measured as the total locomotor activities in I-shaped maze, each terminal of which was equipped with a low-profitability feeder of variable intervals. Localized lesion experiments and single unit recording in freely behaving condition revealed striatal and pallial (isocortical) structures controlling the impulsiveness and work investment. Effects of social competition on these neural substrates will be discussed.

Social environment influences performance in a cognitive task in natural variants of the foraging gene. PS IVa.3

Nancy Kohn (LEGS, CNRS), **Christopher Reaume, Marla Sokolowski** (U. of Toronto at Mississauga) & **Frederic Mery** (LEGS, CNRS)

The importance of how social environment affects behavior has recently received increased attention. In Drosophila, natural genetic variation in the foraging gene, which encodes for a cGMP-dependent protein kinase (PKG), affects the foraging activity of larval and adult flies. Sitters (*fors*) tend to be more sedentary and aggregate within food patches whereas rovers (*forR*) have greater movement within and between patches of food. Additionally, rovers and sitters vary in their performance on a number of cognitive tasks. We hypothesized that these variants would also differ, in a classical olfactory conditioning test, depending on whether they were in groups or alone. Individual performance was affected by PKG activity. In sitters, but not in rovers, the acquisition of information was facilitated by the social interaction (being in a group). In rovers, but not in sitters, the type of social interaction (being with other rovers or with other sitters) affected learning and memory. Also, naïve individual rovers tended to follow groups of conditioned sitters but not groups of conditioned rovers. Our results suggest that for mediates some social aspects involved in learning and memory in Drosophila melanogaster. Also, the traditional way of studying olfactory learning with groups may not be a good indicator of individual performance.

Neuronal correlates of colony recognition cues in ants. PS IVa.4

Andreas S. Brandstaetter, Wolfgang Rössler (University of Würzburg) & **Christoph J. Kleineidam** (University of Konstanz)

Ants discriminate colony members (nestmates) from members of different colonies (non-nestmates) by their colony odor, which consists of colony-specific hydrocarbons on the cuticle (label). During the recognition process, the label is compared to a neuronal template (label-template matching) and a mismatch will lead to aggression. The neuronal basis of label-template matching is unknown. In search for the neuronal correlate of the label, we retrogradely stained output neurons of the primary olfactory center of the ant brain, the antennal lobe (AL), and presented label-treated dummies. We measured neuronal activity in the upper half of the AL in response to both nestmate and non-nestmate label using calcium imaging with a CCD camera. Our results show that workers can perceive the label of their own colony. A template implemented simply as a sensory filter in the antenna, where olfactory receptor neurons are adapted to the ever-present nestmate label and only non-nestmate label is detected, is hence unlikely to be the main mechanism of nestmate recognition. Alternatively, sensory information might be specifically modified along the olfactory pathway to allow discrimination, with specific modifications that result in a template. We compare neuronal activity patterns elicited by nestmate and different non-nestmate labels using multi-variant statistics in search for specific modifications of neuronal representations. In order to map the neuronal activity in the whole AL, we use calcium imaging with a two-photon microscope and analyze the responses of glomeruli located in the lower part of the AL. Differences in the neuronal responses to the label in different parts of the AL might indicate that different mechanisms are realized in parallel to achieve efficient and reliable nestmate recognition.

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Modulation of learning by an alarm signal in an insect brain: evidence for an opioid-like pathway.

PS IVb.1

Elodie Urlacher, Isabelle Massou, Martin Giurfa, Bernard Francés & **Jean-Marc Devaud** (CRCA)

Cognitive processes can be modulated by many factors, including social signals from conspecifics. In social insects, pheromones are such signals that contribute to organize the life of the colony by modulating the behavior and physiology of individuals. Using the honeybee as a model organism to study the neural bases of learning, we show that appetitive olfactory learning is modulated by a particular social experience, exposure to the sting alarm pheromone. After exposure to this signal (released by other bees in life-threatening situations), bees perform less well in a Pavlovian conditioning assay, compared to unexposed bees. The same result can be obtained after exposure to the main component of the pheromone, isopentyl acetate (IPA). Thus, exposure to IPA may trigger some neuromodulator(s) that modify the function of the brain centers known to be involved in this learning task. Pharmacological treatments combined with the exposure to IPA prior to conditioning show that an agonist (fentanyl) and an antagonist (naloxone) of mammalian opioid receptors can mimick or reverse, respectively, the modulation of learning. This suggests that exposure to the alarm pheromone triggers a signalling pathway that shares common features with the mammalian opioid-system. This hypothesis was already proposed, as IPA exposure also can modulate sensitivity to a noxious stimulus (Núñez et al. 1998). However, the existence of an opioid-like pathway was never clearly demonstrated in Invertebrates. We will present molecular and pharmacological data indicating that such a pathway exists in Insects. Our results provide evidence in favour of functional similarities between at least one or two neuropeptides in the honeybee, and some mammalian opioids. We argue that the action of such neuropeptides may be part of an adaptive physiological response to noxious and alarm/stress signals in the honeybee.

Behavioral and cellular bases of vasotocin modulation of agonistic behavior in two species of weakly electric fish. PS IVb.2

Rossana Perrone, Omar Macadar & **Ana Silva** (IIBCE; Fac. Ciencias, Montevideo)

Social behavior in vertebrates is controlled by a complex and conserved brain network. Inter and intraspecific modulations of behavior are correlated with distinctively distributed patterns of activity in this network, which depend on neuroendocrine messengers that integrate extrinsic and intrinsic cues. The neuropeptide arginine- vasotocin (AVT) and its mammalian homologue, vasopressin, are key integrators underlying interspecific, sexual, individual, and social context differences in behavior across vertebrate taxa. Comparative studies of AVT actions between related species with different social structure have been useful for the understanding of the neural bases of behavior. We used two species of weakly electric fish with different social strategies: *Gymnotus omarorum*, territorial and highly aggressive, and *Brachyhypopomus gauderio*, gregarious and only aggressive during the breeding season. We focused on electric organ discharge (EOD) rate modulations, which is one of the components of social behavior in electric fish. AVT produces an immediate and sustained increase of the EOD basal rate of B. gauderio, and has no effect on the basal EOD rate of *G. omarorum.* These results suggest that AVT acts directly on the pacemaker nucleus (PN) in *B. gauderio* but not in *G. omarorum.* Anatomical differences in AVT neurons and projections to the PN were explored in both species. Vasotocin-positive neurons somata in the POA of both species were found. Only *B. gauderio* showed vasotocin- positive fibers projecting to the PN. Submissive electric displays (chirps and interruptions) are produced by subordinate fish during agonistic behavior in both species. These social electric signals depend on pre-pacemaker inputs to the PN. The administration of AVT to putative subordinates in dyadic agonistic encounters allowed us to discriminate the action site of AVT in the modulation of EOD rate in this species.

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Symposium 13

"An integrative theoretical view: the neuroethological approach to computational neuroscience"

Organizer: **Jose L Peña** (Albert Einstein College of Medicine)

Correlations and predictions in the electrosensory system. S 13.1

Leonard Maler (University of Otawa)

Spike trains of nearby neurons are often correlated due either to common input (signal correlations) or correlated noise; equally important are temporal correlations across interspike intervals (ISIs) of one neuron. In the electrosensory system spatial correlation of electroreceptor discharge is an increasing function of AM frequency and high frequency signals can be extracted by a decoder with a large receptive field and high spike threshold. Temporal correlations across ISIs and matched short-term synaptic dynamics enhance the detection of weak low frequency signals.

What can songbirds teach us about how and why we perceive pitch, timbre and rhythm?

S 13.2

Frederic Theunissen (University of California at Berkeley)

We have used a combination of neuro-ethological and classical auditory neurophysiological approaches to study how behaviorally relevant sounds are processed in the avian auditory system. One of the contributions of the neuro-ethological approach in auditory science has been the discovery of highly specific neurons that appear to be specialized for detecting for the detection of a few (or sometimes a single) behaviorally relevant sounds. On the other hand, many auditory neurons recorded in the auditory system of non-specialists do not exhibit such specificity. At the same time, animals and humans hear and process a large space of sounds and are able to categorize these in much broader perceptual terms, describing them in terms of their pitch, timbre and rhythm. By systematic analyzing neural responses to song in the ascending avian auditory system and relating receptive fields to the statistics of natural sounds, we have shown that these two approaches can be unified: we found that the spectro-temporal receptive fields (STRFs) of auditory neurons tile a subset of the acoustical space that is particular important for natural sounds. In addition, we found that neurons could be classified into functional clusters. Neurons in different clusters were sensitive to different song features and could be involved in mediating distinct perceptual attributes.

We then hypothesized that auditory perceptual dimensions (and their neural underlying representations) have evolved because they allow the detection of the information bearing structures in sounds that are robust to distortions and environmental noise. We have tested this hypothesis by analyzing the response of avian auditory neurons to songs embedded in noise. We found that neurons in secondary auditory areas could show a high degree of invariance responding similarly to songs presented in isolation and to songs embedded in noise. This invariant property was correlated to the shape of their functional class as determined by their STRF. These high-level auditory neurons are therefore well placed to provide a reliable signal to brain regions involved in the recognition of specific songs.

The statistics of motor performance during sonar-guided approach determine echo-delay tuning curves in echolocating bats S 13.3

Emilio Salinas (Wake Forest University School of Medicine)

Across organisms and sensory modalities, theories that attempt to understand the responses of sensory neurons typically combine two fundamental ingredients: stimulus statistics (how common is each stimulus) and efficiency criteria (how to transmit the largest amount of information at the lowest possible cost). Thus, theoretical analyses based on concepts such as redundancy reduction, decorrelation, or sparseness have been quite successful at explaining salient properties of various types of sensory neurons. I will discuss a complementary point of view in which the responses of a neuronal population to a sensory parameter x are evaluated in terms of their capacity to drive or guide a motor variable y. The idea is that optimal sensory representations should take into account not only the statistics of the sensory world, but also what the organism does with that sensory information; that is, the statistics of the downstream motor activity that generates behavior. In the case of echolocating bats, reasonable assumptions can be made about those statistics, and how they relate to a key sensory variable: target distance (or echo delay). This framework predicts, from first principles, distinctive features of echo-delay tuning curves; and in general, it suggests that knowledge about the downstream impact of sensory representations is crucial for understanding some of their fundamental properties.

An auditory computational map. S 13.4

Jose L Peña (Albert Einstein College of Medicine)

The owl exhibits a characteristic orienting response towards sound sources, its behavior is highly reproducible and readily measured, the variables involved in triggering specific behaviors are well characterized, and it affords the ability to probe the neural mechanisms involved in the computation at different levels. Whereas spatial selectivity of neurons in the owl's auditory system is broad and ambiguous in low-order structures, it progressively increases along a hierarchically organized pathway. In the midbrain, a map of auditory space is computed based on the differences in time and intensity of acoustic signals that arrive at each ear. These binaural cues are processed in parallel pathways that converge on space-specific neurons. Different regions of the owl's brainstem are crucial for this synthetic process and match predictions made by studies of sound localization in humans. Thus owls, as auditory specialists, offer an opportunity to examine whether optimization leads to mathematical elegance in neural computations.

Symposium 14

"Trichromacy and beyond: colour vision from molecules to perception"

Organizers: **Almut Kelber** (Lund University) & **Natalie Hempel de Ibarra** (University of Exeter)

Colour vision from the periphery to the central bumblebee brain: what about timing?

S 14.1

Angelique Paulk (University of Queensland) & **Wulfila Gronenberg** (University of Arizona)

The visual scene is comprised of numerous visual cues which animals must process to be able to perform behaviors relevant to the context. One major feature of the visual scene is color, which animals use in a variety of contexts, including foraging for food, mating, and social communication. However, color information can complicate the issue, in that it is closely tied with pattern and motion information. To better understand how the visual system can process color information, we recorded from a total of 162 neurons in the bumblebee visual system while presenting color cues. Bumblebees have trichromatic color vision and are well-known for their visual capabilities (Ney-Nifle et al., 2001; Peitsch et al., 1992). In pursuit of understanding how color vision operates in the bumblebee brain, we recorded from neurons at multiple stages of the visual system, from the periphery to the central brain and compared the responses to color stimuli. First, we found that the bumblebee visual system appeared to have segregated parallel pathways for color versus motion processing (Paulk et al., 2008). Upon further examination, we also found that there appeared to be a progression of increasingly complex responses to color information in the neurons from the periphery to the central brain. Finally, we also found that the spike timing could better reflect the color of the stimulus, not just the number of spikes in response to the stimuli. These results could indicate that color information is encoded in the spike times as well as a rate code.

Ney-Nifle M, Keasar T, Shmida A (2001) Location and color learning in bumblebees in a two-phase conditioning experiment. J Insect Behav 14:697-711.

Paulk AC, Phillips-Portillo J, Dacks AM, Fellous JM, Gronenberg W (2008) The processing of color, motion, and stimulus timing are anatomically segregated in the bumblebee brain. J Neurosci 28:6319-6332.

Peitsch D, Feitz A, Hertel H, de Souza J, Ventura DF, Menzel R (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170:23-40.

Perception of color, lightness and polarization in foraging *Papilio* butterflies.

S 14.2

Michiyo Kinoshita (Sokendai-Hayama)

Papilio butterflies discriminate wavelength, intensity and polarization angle of visual stimuli. Their visual organs, the compound eyes, contain six classes of spectral receptors (UV, V, B, G, R, BB), each having its characteristic polarization sensitivity. For example, the UV and R receptors are respectively sensitive to the vertical and diagonal polarization. How does such eye organization affect the vision of *Papilio*? By measuring wavelength discrimination ability of *Papilio*, we found that their color vision was tetrachromatic. We trained a *Papilio* to feed at a monochromic light and let it select one from two monochromatic lights. We thus found that they could detect 1 nm difference in the wavelength region around 430, 480, 560 nm. Simulation using the noise-limited color opponency model revealed that the color vision was based on the UV, B, G and R receptors. *Papilio* butterflies also perceive lightness contrast. From two identical color disks each presented on bright or dark gray, the butterflies selected one on dark gray. This is due to their innate preference to brighter stimulus, and also indicates that they perceive lightness contrast. We also found that foraging *Papilio* butterflies innately preferred vertical (V-pol) to horizontal polarization (H-pol). The preference to V-pol was suppressed when V-pol on light gray and H-pol on dark gray presented side by side: they preferred H-pol, as if H-pol appeared brighter than V-pol due to lightness contrast. We therefore concluded that foraging *Papilio* detected V-pol as a brighter stimulus than H-pol. Most likely, *Papilio* uses the UV/B/G/R system for discriminating polarization as well as lightness, because none of these receptors have high sensitivity to H-pol, which is therefore less effective to the system.

High and low level mechanisms in bird colour vision. S 14.3

Daniel Osorio. (University of Sussex)

Animals use colour to recognise objects, and to make judgements about the quality of food sources potential mates and so forth. We often have a good knowledge of photoreceptor spectral sensitivities that ultimately limit the ability to discriminate visible spectra, but human colour vision entails much more than simple discrimination; we recognise different aspects of colour such as hue, saturation and brightness, and classify colours by name. Given this rich experience, it is natural to ask about the evolutionary and ecological relevance of human colour vision, and conversely how far our abilities are shared by other species. Birds have four types of cone visual pigment and tetrachromatic colour vision. I will explain how simple physiological models and acknowledge of photoreceptor properties allow us to investigate 'higher level' visual abilities - and perhaps the underlying mechanisms - involved in colour vision and object recognition of other animals. We find that young poultry chicks learn colour accurately, perhaps reflecting a reliance on spectral information over form and pattern. Similarly, poultry chicks make sophisticated use of colour variation within signals to make decisions about novel objects and to learn from the outcomes of these decisions.

Curing color blindness in an adult primate using gene therapy. S 14.4

M. Neitz, K. Mancuso, J. Neitz (University of Washington)

Red-green color blindness, which results from the absence of either the long- (L) or middle- (M) wavelength-sensitive visual photopigments, is the most common single locus genetic disorder. The possibility of curing color blindness using gene therapy was explored by adding a third type of cone pigment to retinas of dichromatic squirrel monkeys. The addition of a third opsin gene in adults was sufficient to produce trichromatic color vision. Classic visual deprivation experiments have led to the expectation that neural connections established during development would not appropriately process an input that was not present from birth. If true, then treatment of congenital vision disorders would be ineffective unless administered to the very young. Here, however, addition of a third opsin in adult red-green color-deficient primates was sufficient to produce trichromatic color vision behavior. Thus, trichromacy can arise from a single addition of a third cone class and it does not require an early developmental process. This opens a new avenue to explore the requirements for establishing the neural circuits for a new dimension of color sensation.

Symposium 15

"Networks for communication: Between human, ape. Monkey and bird brains"
Organizer: **Chris Petkov** (Newcastle University)

Learned birdsong and the neurobiology of human language. S 15.1

Erich D. Jarvis (Duke University Medical Center)

Here I will present the known and hypothesized similarities and differences in the brain and behavior for vocal learning in songbirds and humans. I will also discuss how vocal learning is critical behavioral substrate that makes 'spoken language' special. Of the vocal learning species whose brains have been studied to date (songbirds, parrots, hummingbirds, and humans), all have been found to have a forebrain to brainstem system that controls vocalizations, whereas those species that produce only innate vocalizations have only the brainstem vocal system. Using behavioral, molecular, electrophysiological, and anatomical approaches we found that the song learning systems of the three distantly related vocal learning birds are each embedded within a motor system involved limb and body movements. This motor system is also present in vocal non-learning animals, but without the presence of song learning system embedded within it. The song learning and adjacent motor systems share many properties in common, including motor-driven gene expression cascades and neural network architecture necessary for motor learning. These pathways have parallels with those for spoken language in humans. A computational analysis of genomes of 23 mammalian and 2 avian species, including vocal learners (humans, bats, dolphins, elephants, and songbirds) versus vocal non-learning mammals (e.g. chimpanzees, macaques, dogs, cows, an chicken) suggest the presence of unique mutations in axon guidance molecules, particularly for motor pathways, in vocal learners. To explain these findings, I propose a motor theory for the origin of vocal learning and other complex behavioral traits. Much like gene evolution, I propose that an ancient brain system used to control movement and motor learning, such as learning how to walk, fly, or gesture, duplicated and then diverged with axon guidance mutations to directly connect to the brainstem motor neurons that normally control innate vocalizations, but now song or spoken language. The pre-existing system, I argue, is a fundamental design of the vertebrate brain, consisting of the two motor sub-pathways (anterior and posterior), which during embryonic development form parallel systems to control different muscle groups that are innervated by sensory systems for feedback control of different motor behaviors. In this manner, the evolution of brain pathways for vocal learning may have evolved independently of a common ancestor in distantly related birds and in humans, but dependent on a pre-existing genes and a pre-existing motor learning pathway with deep homologous roots.

Rethinking Wernicke's Area: Pathways and hierarchies of speech comprehension in the human brain. S 15.2

Jonas Obleser (Max Planck Institute for Human Cognitive and Brain Sciences)

Auditory language comprehension is a complex cognitive process that is accomplished by the human brain in a surprisingly robust and efficient manner, despite countless possible adversities. Presenting own as well as reviewing other recent evidence from functional human neuroimaging (fMRI, EEG) and behaviour, the case for a highly dynamic network of superior temporal as well as heteromodal extra-temporal brain areas in speech comprehension will be made. I will ask and propose tentative answers to

(i) How do the central auditory pathways deal with noise at the signal entry level in speech comprehension?
(ii) Which are key contextual influences that aid speech comprehension when the signal quality drops?
(iii) What is the functional circuitry within and across auditory cortex that copes with such speech comprehension difficulties?

Here, the role of the *Planum Temporale*, a core area of what is known as "Wernicke's area", is currently undergoing particular redefinition, and I will present a quantitative meta-analysis to show that the Planum Temporale is particularly suited to support effortful speech comprehension under noisy conditions.

Do monkeys have anything like the speech and voice regions in the human brain? S 15.3

Christopher I. Petkov (Newcastle University)

Traditionally, neurobiological work on human communication has focused on the unique aspects of human speech perception and the brain processes that support it. More recently, human brain imaging ('neuroimaging') work has evaluated the 'stimulus-bound' aspects of speech and voice processing, which better relate to the processing of communication sounds in other animal species. To obtain new insights into the relationship of the systems processing communication signals in primates, we summarized the results from several neuroimaging studies in humans, chimpanzees and macaques. While some differences across the species remain, our observations suggest a general correspondence in how the primate temporal lobes analyze species-specific vocalizations. A closer and more striking correspondence is seen in a region in the right anterior-temporal lobe of both humans and macaque monkeys, where sensitivity to the vocal identity of, respectively, different talkers and callers is observed. The accumulating evidence for both anterior and posterior temporal lobe involvement in speech and voice processing in humans now reveals a close relationship to the neuroimaging evidence being obtained in nonhuman primates.

Belin, P., Zatorre, R. J., Lafaille, P., Ahad, P., & Pike, B. (2000). Voice-selective areas in human auditory cortex. Nature, 403(6767), 309-312.

Petkov CI, Kayser C, Steudel T, Whittingstall K, Augath M, Logothetis NK. (2008) A voice region in the monkey brain. Nat Neurosci, 11(3), 367-374.

Taglialatela JP, Russell JL, Schaeffer JA, Hopkins WD. (2009) Visualizing vocal perception in the chimpanzee brain. Cereb Cortex, 19(5):1151-1157.

Communicative signaling and the chimpanzee brain. S 15.4

Jared P. Taglialatela (Kennesaw State University)

The study of nonhuman primate vocal-auditory behavior continues to provide novel insights into the origins of human language. However, data on the neural systems involved in the perception and processing of conspecific vocalizations in great apes are virtually absent in the scientific literature, yet are critical for understanding the evolution of language. Here we used positron emission tomography to examine the neurological mechanisms associated with the perception of species-specific vocalizations in chimpanzees. The data indicate right-lateralized activity in the chimpanzee posterior temporal lobe, including the planum temporale, in response to certain calls, but not others. In addition, important differences are apparent when these data are compared to those published previously from monkey species suggesting that there may be marked differences in the way in which chimpanzees and macaque monkeys perceive and process conspecific vocalizations. These results provide the first evidence of the neural correlates of auditory perception in chimpanzees. These data suggest that not all chimpanzee vocalizations are functionally equivalent, and point to heretofore unrecognized level of complexity in the chimpanzee vocal repertoire

Special Lecture

Franz Huber Founders Lecture

Sten Grillner (Karolinska Institutet)

"The lamprey motor system - from selection of behaviour to mechanisms of pattern generation." SL2

Goal directed locomotion requires not only the generation of the standard motor pattern produced by the CPG networks that are turned on from the brainstem command regions (MLR/DLR), but also a maintained control of body orientation (posture) during the locomotor movements and the control of steering. My presentation, based on the lamprey CNS, will address the intrinsic function of the CPG and particularly the intersegmental coordination (forward-backward locomotion) and its supraspinal regulation. In addition, I will discuss the tectal mechanisms underlying steering movements with the retinotopic motor map, and the control from the output of the lamprey basal ganglia. Furthermore, the mechanism by which different motor programs are selected will be considered with special reference to the basal ganglia – experiments and modelling. Our recent findings establish that the structure and function of the basal ganglia have been conserved to a surprising degree from the ancient lamprey version to mammals. This applies to the input to striatum (pallium, thalamus, dopamine, 5-HT, histamine input), the pallidal structures and their output targets, the cellular properties of striatal and pallidal neurons and the effects of an MPTP induced dopamine denervation.

Kozlov A, Huss M. Lansner A, Hellgren Kotaleski J, Grillner S. (2009) Simple cellular and network control principles govern complex patterns of motor behavior. PNAS 24;106:20027-20032.

Jones, M, Grillner, S., Robertson, B. (2009) Selective projection patterns from subtypes of retinal ganglion cells to tectum and pretectum: distribution and relation to behaviour. J Comp Neurol 517:257-275.

Grillner, S. (2006) Biological Pattern Generation: The Cellular and Computational Logic of Networks in Motion. Neuron 52; 751-766

AUGUST 7

Plenary Lecture 9

Heiner Römer (Karl-Franzens-University)

"Neuroethology of acoustic mate preferences in field crickets: ecological and evolutionary perspectives" PL 9

The auditory behaviour of crickets is a fascinating model for both ethologists and neurobiologists to study fundamental proximate and ultimate questions of behaviour. In particular, female mate preferences have been used in behavioural and evolutionary ecology to document how they contribute to variance in male mating success and generate selection on male traits. At the same time, and often not acknowledged by behavioural ecologists, neuroethological studies have systematically examined the underlying neuronal mechanisms which result in these preferences. In my talk, I make an attempt to integrate these two approaches, by examining aspects of mate preferences and the neuronal coding under various ecological conditions and behavioural paradigms.

First, in a comparative approach on European field crickets and tropical rainforest crickets I show that the strong competition for the airborne sound channel resulted in a sharpening of sensory filters in the latter, enabling to listen to conspecific sound signals under high nocturnal background noise levels of heterospecifics. I also present evidence that such competition resulted in a better match between the two peripheral filters for sensitivity and directionality.

The natural situation in which mating preferences are usually expressed are often characterized by the fact that (1) signals of several mates can be compared either simultaneously or sequentially, (2) females choose their mates based on several attributes, either in the same or in different modalities, and (3) signal detection, evaluation and localization is impaired by constraints of the transmission channel. Behavioural and neurophysiological approaches in the lab and outdoors will highlight some of the consequences for acoustic mate choice in this model organism.

Plenary Lecture 10

Dan Margoliash (University of Chicago)

"Sleep processes in learning and memory." PL 10

Many if not all animals sleep, but we know less about the various neuronal mechanisms associated with sleep than for most other behaviors. One aspect of sleep that is receiving attention is its role in learning and memory phenomena. This also has the advantage that it integrates human and animal work.

A series of recent studies help to define the role of sleep on human performance in a broad range of learning tasks (perceptual, motor, sensorimotor, and cognitive). Several conclusions arise from these studies. One, the patterns of sleep effects are less varied than once believed, once certain features of performance are better defined. Two, there is a robust distinction comparing rote and generalized learning. Three, sleep is an active process where information is likely to be created as well as consolidated. A model system we have recently introduced (starling adult perceptual song learning) shares many of the features observed in humans and holds promise to extend the human observations.

Birdsong learning, a well-known model system for vocal learning, also shows adaptive sleep-associated developmental learning effects. Superimposed over a developmental trajectory of increased song complexity and emerging concordance with the tutor song to be copied, a juvenile's singing also shows a complex daily pattern of variation. This pattern of singing emerges not on the day a bird is first exposed to a tutor song but starting the following morning.

We have the beginnings of an understanding as to the mechanisms associated with this learning phenomenon. Many neurons in the song motor system take on one of two modes, regular or bursting. In both adults and juveniles, burst firing is associated with singing during the day but at night can also occur as spontaneous "replay" of singing. The evidence to date suggests that replay carries sensory information, and helps reconfigure the network. This motivates provisional mechanistic models of sleep-dependent learning.

Symposium 16

"The neuroethology of context-dependent locomotion"

Organizer: **Karen Mesce** (University of Minnesota)

The neural substrate for the transformation of olfactory inputs into locomotor output.

S 16.1

Réjean Dubuc (Université du Québec à Montréal et Université du Montréal)

Motor behaviors, including locomotion, are generated by olfactory inputs in different behavioral contexts such as food seeking, social communication, and reproduction. The stereotyped nature of some of the induced motor responses (escape, attack) suggests a strong neural link between olfactory inputs and motor command centers in the CNS. We have used the lamprey model to identify the neural substrate underlying the transformation of an olfactory input into a locomotor output. We found that olfactory stimulation induced large excitatory responses in reticulospinal cells, known to act as command neurons for locomotion. The olfactory inputs were relayed in the medial part of the olfactory bulb and projected to the posterior tuberculum in the ventral diencephalon. From there, the signal was sent to the mesencephalic locomotor region to eventually reach reticulospinal cells in the lower brainstem. Activation along this olfactory-motor pathway generated rhythmic ventral root discharges as well as swimming behavior. The discovery of a pathway dedicated to the production of locomotor movements in response to olfactory inputs constitutes a first in any vertebrate species. We also found that this pathway is present at all life stages of lampreys. On the other hand, the behavioral responses to pheromones, for instance, are elicited only at specific life stages. Modulatory mechanisms may thus be involved in this olfactory-locomotor pathway. We have recently found that GABA transmission powerfully modulates transmission within the olfactory bulb. The injection of the $GABA_A$ receptor antagonist, gabazine, into the olfactory bulb amplified pre-existing reticulospinal responses to olfactory nerve stimulation. This suggests that GABA inputs filter transmission at the level of the olfactory bulb in lampreys and could thus modulate locomotor output depending on the state of the animal.

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Postembryonic development of locomotor control circuitry in the vertebrate spinal cord. S 16.2

Keith Sillar & **HongYan Zhang** (University of St Andrews)

Adult vertebrate locomotion needs to be inherent versatility to enable animals to navigate efficiently through the environment. The associated underlying motor control networks of the CNS, called central pattern generators, are assembled during embryogenesis before undergoing a period of postembryonic maturation. Once assembled, the same networks are influenced by a range of neuromodulators which confer increased flexibility on motor output in part by influencing the discharge properties and recruitment of spinal motor and interneurons. During this period, the role played by motorneurons in generating locomotion is modified during development to provide for changes in direction and intensity. In post-embryonic *Xenopus laevis* frog tadpoles myotomal motorneurons initially form a relatively homogenous pool normally discharging a single impulse in each cycle of swimming and innervating the majority of dorsoventral extent of a myotome. Over the course of the next day or so of larval life this situation transforms dramatically into one in which motorneurons do not fire on every cycle and can be recruited to discharge multiple impulses in some cycles. These motorneurons have also differentiated into high, medium and low threshold units. The peripheral innervation fields also differentiate during this period to the extent that individual motorneurons now possess predominantly ventral, medial or dorsal innervating fields. We found no correlation between recruitment threshold and innervation field - ventral, medial and dorsal fields each being served by members of high, medium and low threshold motorneurons populations. This unfolding developmental plan equips the organism with the neuronal substrate to bend, pitch, roll and accelerate during swimming, which will be important for survival during the free swimming larval life that is about to ensue.

Development and the functional specialization of spinal locomotor networks.

S 16.3

David McLean (Northwestern University)

For most, if not all, animals the ability to move improves with age. In vertebrates, the generation of locomotor movements by spinal networks is tailored to the morphology and environment of the animal. However, a general pattern that appears to be conserved in developing vertebrates regardless of their propulsive strategy is the emergence of relatively gross body movements that are gradually replaced by more refined ones. Many have focused on the contribution of descending pathways and sensory inputs to the maturation of locomotor movements, but less is known about the contribution of spinal neurogenesis to this process. Here, I will discuss recent evidence from studies of zebrafish (*Danio rerio*), which indicate that progressive improvements in locomotor abilities are due, at least in part, to the sequential addition of spinal interneurons during development. Neurons that are born earliest ultimately control movements in zebrafish larvae that allow them to escape predators, with neurons that control the more refined movements required for hunting added later on as the zebrafish grows. A consequence of the systematic addition of spinal interneurons in zebrafish is a topographic map in the larval spinal cord that represents when the cells developed and when they are recruited during different speeds of swimming, which ultimately reflects the behavioral context in which these spinal networks are activated. I will discuss the relevance of these findings to the organization of motor systems in general.

Dopamine and the selection of locomotor form in a simpler neural system.

S 16.4

Karen Mesce (University of Minnesota)

In the leech, the decision to crawl or swim is dependent on the internal neuromodulatory state of the animal. We determined that dopamine biases locomotion in the direction of crawling while inhibiting swimming. Individual cephalic neurons have been identified that can elicit crawling or swimming, but this outcome too is based on context, i.e., the environment surrounding the leech. How such environmental and neurohormonal factors influence cephalic cells and shape the output of iterated crawl pattern generators will be presented.

The graceful beauty of an animal in motion often reflects the neural choreography of underlying oscillator circuits and their coordination with each other. The fluidity of locomotor activity, however, depends on the correct decision to perform appropriate movements within the confines of an animal's environment. For example, the medicinal leech is an elegant swimmer in water, but not on land. In contrast, in the context of a terrestrial environment, the leech shows effective and equally graceful locomotion by way of crawling, an especially flexible behavior that consists of repeated whole body elongation and contraction phases. During each of these phases, a series of anterior-to-posterior metachronal waves of motor activity propel the animal in a forwards-only direction. Recently, we established that each of the 21 body segments of the leech contains a central pattern generator (CPG) controlling both phases of crawling. We established that dopamine (DA) can activate the crawl CPG in a single ganglion or entire nerve cord. Crawling is not only activated by DA, but swimming is inhibited and no stimulus can turn the swim oscillators on. We also determined that the compound cephalic ganglion is both necessary and sufficient for the expression of spontaneous or evoked crawling, but not swimming. If the isolated CNS is biased by DA to exhibit fictive crawling, subsequent (reversible) removal of the cephalic ganglion results in the loss of inter-oscillator isochronicity and appropriate phase delays. To identify specific neurons in the brain important for the activation and coordination of crawling, we targeted a previously studied cephalic neuron, R3b-1. This long-distance-projecting neuron was shown to activate crawling or swimming in nearly-intact leeches if their bodies were placed in either shallow or high fluid levels, respectively. In the isolated CNS, electrical stimulation of R3b-1 gates either swimming or crawling unpredictably, but after DA perfusion this cell activates crawling exclusively. In DA, cell R3b-1 shows large slow waves that are phase-locked to the crawl rhythm. Furthermore, the frequency of crawl motor bursts is correlated with the amount of current injected into R3b-1; additional cell manipulations alter inter-oscillator coordination. These data suggest that R3b-1 is a DA-sensitive neuron vital for the activation and coordination of the individual crawl oscillators. Curiously, if the cephalic ganglion and nerve cord are disconnected, overt crawling behavior is lost but begins to return after about 1-2 weeks, a gain of function independent of nerve cord regeneration. In these animals, unnatural backwards crawling is sometimes observed.

Symposium 17

"Computational Mechanisms in Temporal Processing"

Organizer.- **Bruce Carlson** (Washington University at St. Louis)

The grasshoppers' solution to computational problems in temporal processing. S 17.1

Bernhard Ronacher (Humboldt Universität zu Berlin)

Many grasshoppers use acoustic signals to attract and assess potential mates. Acoustic communication constitutes an important barrier against hybridization events and the resulting loss of reproductive success. For signal recognition, grasshoppers rely primarily on the temporal pattern of amplitude modulations (AM). In this recognition task, however, they face several computational problems: (i) noise problems, (ii) resolution problems, and (iii) invariance problems. A serious invariance problem results from the large temperature differences that may exist in a meadow. In these ectothermic animals, the speed of stridulation movements depends strongly on ambient temperature, and the duration of AM pattern elements my change by a factor two or more. We present a simple solution how a neuronal recognition mechanism may cope with this temperature dependent time warp of the signals.

The resolution problem is connected with a noise problem. Auditory neurons exhibit a substantial trial-to-trial variability in their spike trains, which results from a multitude of stochastic processes involved in the generation of the neuronal signals. However, their small auditory system lacks the option to average across hundreds of neurons to reduce this 'intrinsic' noise. We asked how relevant this intrinsic noise may be in relation to the external degradation the communication signals experience on their way between sender and receiver. One grasshopper species is able to detect 2-ms gaps in noise stimuli – in this respect these insects are not inferior to vertebrates. Nevertheless, this gap detection capacity seems at odds with the trial-to-trial variability of spike trains and with corner frequencies below 100 Hz derived from modulations transfer functions.

Temporal pattern recognition through short-term synaptic plasticity.

S 17.2

Bruce Carlson (Washington University in St. Louis)

Mormyrid electric fish are timing specialists. They communicate using a brief pulse of electricity referred to as an electric organ discharge (EOD). The waveform of the EOD conveys information about the species, sex and status of the sender. In addition, mormyrids are able to generate a rich repertoire of electric displays by varying the interpulse intervals (IPIs) between EODs. These electric communication signals are encoded by peripheral receptors into precisely-timed spikes: differences in spike timing between receptors code for EOD waveform, whereas sequences of interspike intervals within receptors code for IPIs. This talk will focus on the processing of IPIs, using the mormyrid electrosensory system as a model for understanding how neural circuits can detect different temporal patterns of presynaptic input. A specific population of midbrain electrosensory neurons exhibits tuning to IPI: some of these neurons respond selectively to long intervals (low-pass tuning), others respond selectively to short intervals (high-pass tuning), while still others respond selectively to a limited range of intermediate intervals (band-pass tuning). During presentation of continuously varying IPIs, the responses of many of these neurons also differ depending on whether IPIs are increasing or decreasing. Playback of natural IPI sequences recorded from freely-behaving fish reveals that these temporal tuning properties result in individual neurons that selectively code for specific communication displays. The strong selectivity of these neurons for particular temporal patterns of input appears to relate to a dynamic interplay between summation and short-term synaptic plasticity in excitatory and inhibitory pathways. Rate-dependent variation in the balance between excitation and inhibition may represent a general mechanism for the processing of behaviorally-relevant stimulus information encoded into temporal patterns of activity by sensory neurons.

Mechanisms for generating filters of temporal information in acoustic signals.

S 17.3

Gary Rose (University of Utah)

The temporal structure of acoustic signals plays important roles in animal communication, including human speech. Examples of temporal information include patterns of frequency modulation and amplitude modulation, including the duration and shape of temporally discrete elements e.g., sound pulses, and the intervals between successive elements. Temporal information is coded in the spatio-temporal patterns of discharges in the auditory periphery, but recognition and discrimination of particular temporal information requires that these patterns be read. The anuran auditory system is one of several neuroethological systems that have played important roles in revealing how temporal information is represented and processed in the central nervous system. This work relates broadly to a fundamental question in Neuroscience; how are temporal patterns of spike activity interpreted in the CNS? Several decades of research on the auditory systems of anurans and other animals have revealed neurons that respond selectively to particular temporal features, and thereby decode temporal patterns of afferent activity. The mechanisms that underlie these selectivities, however, have largely remained unknown. Insights into mechanisms are now beginning to emerge, aided by new techniques for using patch-type pipettes to make 'whole-cell' (intracellular) recordings from central neurons, in vivo. I will present recent data from whole-cell recordings in anurans that demonstrate how excitation, inhibition and synaptic plasticity can be integrated in diverse ways to create selectivity for temporal properties such as pulse duration, interpulse interval and interval counting.

Cortical processing during behaviour. S 17.4

James Poulet (Max-Delbrück-Center for Molecular Medicine, Berlin)

Internal brain states form key determinants for sensory perception, sensorimotor coordination and learning. A prominent reflection of different brain states in the mammalian central nervous system are the distinct patterns of cortical oscillations and synchrony, as revealed by extracellular recordings of the electroencephalogram, local field potential and action potentials. Such temporal correlations of cortical activity are thought to be fundamental mechanisms of neuronal computation.

I will present data using single and dual whole-cell recordings from primary somatosensory barrel cortex in mice performing whisker behaviour to examine the cellular correlates of cortical state change. The membrane potential of nearby neurons undergo slow large amplitude fluctuations that are highly correlated during quiet wakefulness, but when the mouse is whisking, cortical neurons depolarise and undergo a state change that reduces the membrane potential fluctuations as well as the correlation between nearby neurons, resulting in a desynchronised local field potential and electroencephalogram.

Cortical state change persists after cutting the primary sensory nerve from the whisker pad and therefore is generated internally, within the CNS. Juxtacellular recordings from thalamic neurons during whisker movements reveal an increase in thalamic spiking activity during whisker movements. Inactivation of thalamus, by thalamic injection of muscimol, increases cortical slow fluctuations during quiet periods. During active periods, when the mouse is whisking, cortical neurons become hyperpolarised during whisking, in contrast to the depolarised state during whisking under control conditions, and the slow fluctuations disappear. The thalamus is therefore responsible for one key aspect of the cortical state change - it appears to provide the tonic depolarising input during whisking.

Symposium 18

"Coupled Robot-animal systems"

Organizer: **Malcolm A. MacIver** (Northwestern University)

Towards analysis of rodent behaviour in large and complex environments.

S 18.1

Hanspeter A. Mallot, Philipp Schwedhelm and **Johannes Thiele** (University of Tübingen)

Despite extensive work on rat behaviour in laboratory tasks, knowledge of behaviour in natural and large environments is still rather limited. In this talk, I shall present two methodological approaches for assessing large-scale and subterranean behaviour, respectively. For large scale environments, we have developed a virtual reality device consisting of a running ball (omnidirectional treadmill) with position tracking and a panoramic projection screen. Visual feedback to translational movements is provided by a virtual reality software. The rat is free to turn on the spot, i.e. rotations are done in closed loop. Behavioural experiments indicate that the rat can be trained to explore and use large scale environments and to proceed to remembered places. For the tracking of rats in subterranean environments, we are exploring sensor-mode technology. In a series of preparatory studies, we have deveoped systems for monitoring rat locomotion (using accelerometers) and vocalizations. Both systems are optimized for minimal energy consumption and memory requirements. The current state of the project will be illustrated by means of laboratory experiments.

How to talk to the mind of the hive? Collective self-organized behavior in mixed groups of robots and animals. S 18.2

José Halloy & Jean Louis Deneubourg (Université libre de Bruxelles)

Understanding how to control self-organized collective behavior is of interest in biology either to study collective animal behavior or to manage group living animals. It is also of interest for engineers designing collective intelligence to control self-organized collectives of devices. Here, we explore theoretical and experimental models for modulated collective behavior in mixed groups of robots and animals. We present results on shelter selection by robots and cockroaches and flocking and decision making in mixed groups of chicks and robots. Replacing some individuals by robots allows probing hypothesis about the role of social interaction in collective decision-making as the behavior of the robots is easier to modulate. The first challenge is to establish a communication link between the robots and the animal i.e. to make them accepted as being part of the group. We present two examples, one based on chemical communication with cockroaches and another based on filial imprinting with chicks. The advantage of autonomous robots are: (i) closing the loop of interaction between the animal and the robots used as responsive elaborate lures; (ii) allowing repetitive social interactions as animal do; (iii) build and test formal behavioral models linking individual cognitive capabilities with social behavior, (iv) studying collective or distributed animal cognition.

Physiologically relevant sensory feedback for prosthetic limbs: Investigations of the neural mechanisms of functionality, cortical reorganization, and perception of limb ownership.

S 18.3

Paul Marasco (Advanced Platform Technology Center, LSCVAMC), **Aimee Schultz** (Neural Engineering Center for Artificial Limbs, RIC), **Keehoon Kim** (Cognitive Robotics Center, Korea Institute of Science and Technology), **Edward Colgate** (Dept. of Mechanical Engineering, Northwestern University), **Michael Peshkin** (Dept. of Mechanical Engineering, Northwestern University) & **Todd Kuiken** (Neural Engineering Center for Artificial Limbs, RIC)

Here we describe research integrating robotic artificial limbs with the sensory nervous system of human amputees who have undergone a nerve redirection surgery. The sense of touch is essential to intuitive limb control yet prosthetic arms do not provide amputees with sensory feedback. Targeted Reinnervation (TR) was developed as a neural-machine-interface to improve prosthetic limb function. Arm nerves left after amputation are surgically redirected to new muscle and skin sites to create neural interfaces for sensory feedback and limb control. These amputees feel a touch to the reinnervated target skin like it is a touch to their missing hand. TR provides a unique standpoint to study the mechanisms of neural plasticity and sensory integration. Brain recordings in rats with amputation and TR revealed that redirected nerves established an expanded representation in sensory cortex. Evoked potential latencies suggested direct connection of the redirected afferents to the forelimb processing areas. Rat receptive fields show similarities to human amputee touch percepts suggesting sensations were perceptual manifestations of cortical reorganization. Psychophysical tests in human TR amputees provide evidence that the target skin acquired a level of tactile acuity approaching that normally found in the hand and fingers. Having a better understanding of these neural substrates has provided a means to capitalize on mechanisms of sensory integration and we have found that self-reported, psychophysical and physiological measures of limb ownership indicated that returning physiologically appropriate cutaneous feedback from a prosthetic limb can drive a perceptual shift towards embodiment of the device. These results suggest that neural mechanisms are in place to functionally maximize physiologically appropriate touch feedback and may help amputees regain a more intact self-image, allowing them to see a prosthetic as less of a tool and more like a part of their own body.

Using robotics to close the loop. S 18.4

Michael Dickinson, Andrew Straw & **Peter Polidoro** (Caltech)

With rare exceptions, the motor actions of animals are likely to generate changes in the stream of sensory information that they receive. Such motor-generated changes in sensory input range from the simple exafferent feedback created as a consequence of motion through the environment to the complex stimuli elicited when one animal elicits communication from another. Presumably, nervous systems have evolved in a way that reflects the intrinsic closed-loop nature of motor output and sensory input. The vast majority of experiments in system neuroscience, however, are performed under open loop conditions, in which an animal's behavior does not alter the sensory input that it receives. Although such protocols are useful precisely because they remove exafferent feedback and thus permit characterization of sensory-motor pathways, real world behavior is often difficult to predict from open-loop performance. In our presentation, we will describe several ways in which real-time feedback is used to create flexible experimental systems that permit the quantitative analysis of sensory-motor behaviors under both open- and closed-loop conditions. The examples will come from behavioral studies of the fruit fly, Drosophila, with questions ranging from flight control to visually-mediated social interactions. In one example, a real-time machine vision algorithm is used in conjunction with active visual display to study flight control in free flying animals. By altering visual display according to an animal's instantaneous position, it is possible to create a full blown virtual reality environment for a free flying fly. In another example, we couple a walking fly's behavior to the motion of a simple robotic fly 'avatar', controlled by a system of servo motors. By moving the robotic fly within a frame of reference determined by a real fly, it is possible to accurately characterize visually-mediated social interactions. Because of advances in machine vision and micro actuation, real-time experimental systems should be come more widely available in the near future.

ABSTRACTS

from

POSTERS (P)

SESSION I (P 1-195) (August 3 and 4)

and

SESSION II (P 196-399) (August 5 and 6)

POSTER SESSION I (August 3 and August 4)

Anatomical Approaches

421 **P 1**

Bursicon expression in adult insects may reveal a division between holometabolous and hemimetabolous insects.

Bursicon is a 30 kDa heterodimeric cystine knot neurohormone that regulates sclerotization of the soft cuticle after ecdysis as well as wing inflation after eclosion. Given this functional role, bursicon should not be needed in adult insects. Experiments in *Drosophila* (Diptera: Brachycera) and *Manduca sexta* (Lepidoptera) support this conclusion, as the bursicon producing cells in the abdominal ganglia of these two insects die soon after emergence. Data in Drosophila suggest that bursicon plays a role in the apoptosis of these neurons. Using RT-PCR and immunocytochemistry we describe the developmental expression pattern of bursicon in Anopheles gambiae (Diptera: Nematocera), and show that in this insect species bursicon expression also ceases following ecdysis. However, in *Periplaneta americana* (Blattaria) and *Teleogryllus commodus* (Orthoptera) bursicon is expressed in the abdominal ganglia of adults. Interestingly, the three species in which the neurons die in adults are holometabolous whereas the two species in which they do not are hemimetabolous. Additionally, the three holometabolous insects are soft bodied and have relatively short life spans, while *P. americana* and *T. commodus* have harder cuticles and live significantly longer. To uncover factors that determine whether or not bursicon is expressed in adult insects, we are currently expanding our investigation to include diverse insects belonging to both the holometabolous and hemimetabolous groups but that have different life histories. These investigations may reveal additional functions of bursicon beyond those already known.

Honegger, Hans-Willi (Vanderbilt University); Shirley , Logan (Vanderbilt University); Estevez-Lao , Tania (Vanderbilt University); Hillyer , Julian (Vanderbilt University)

Allometry and grade shifts drive extreme differences in brain size and proportions between solitarious and gregarious desert locusts

Desert locusts show extreme phenotypic plasticity depending on population density. At low population densities, locusts occur in a cryptic solitarious phase. Crowding triggers the transformation into the behaviourally and morphologically distinct gregarious phase. We investigated whether the pronounced differences in life style are reflected in the size of the brain and the proportional size of brain regions of known function and hence whether phase-related phenotypic plasticity extends to the size and proportions of the locust brain. The total neuropile volume of the brain was 27% larger in gregarious locusts, yet their body weight was 21% lower. The phases showed pronounced differences in brain proportions. These arose from a combination of allometric effects and phase-specific deviations from the allometric expectation. The optic lobe : midbrain ratio was on average 0.89x smaller in gregarious locusts. Gregarious animals had a 1.16x larger medulla than solitarious animals of corresponding lamina size. Moreover, for allometric reasons, they had on average 1.07x more lobula neuropile per unit medulla. Within the midbrain, there was an extreme phase difference in the ratio of the second to the first olfactory neuropile, with gregarious locusts having a 1.41x greater average primary calyx : antennal lobe ratio. Their antennal lobe : brain ratio was 0.87x lower due to non-allometric change, whereas their primary calyx : brain ratio was 1.22x higher but largely in line with the allometric expectation. The accessory calyx and central complex both showed a non-allometric expansion in gregarious brains. Conclusion Changes in population density drive phenotypic plasticity in absolute and relative investment into metabolically costly brain tissue. Solitarious locusts have smaller brains and invest relatively more into low-level sensory processing, possibly to gain sensitivity. Gregarious locusts have larger brains and prioritise higher integration, which may reflect the greater complexity of their environment.

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Are bigger brains better?

Attempts to relate brain size to behaviour and cognition have rarely integrated information from insects with that from vertebrates. Many insects, however, demonstrate that highly differentiated motor repertoires, extensive social structures and cognition are possible with very small brains, emphasising that we need to understand the neural circuits, not just the size of brain regions, which underlie these feats. Neural network analyses show that cognitive features found in insects, such as numerosity, attention and categorisation-like processes, may require only very limited neuron numbers. Thus, brain size may have less of a relationship with behavioural repertoire and cognitive capacity than generally assumed, prompting the question of what large brains are for. Larger brains are, at least partly, a consequence of larger neurons that are necessary in large animals due to basic biophysical constraints. They also contain greater replication of neuronal circuits, adding precision to sensory processes, detail to perception, more parallel processing and enlarged storage capacity. Yet, these advantages are unlikely to produce the qualitative shifts in behaviour that are often assumed to accompany increased brain size.

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Brain scaling in insects: Does being small intensify 'brains vs. brawn' trade-offs?

The size of an animal's brain and nervous system imposes a fundamental limit on its ability to process information and thus its behavioral sophistication. Brain size, in turn, may be constrained by high metabolic demands of neural tissues and by competition for body space with other organs. Most studies of brain scaling have been done on very large or small animals (vertebrates or ants). We studied brain scaling of insects with body volumes in the neglected mid size range of ca. 40 µL to 50,000 µL (~40 to 34,000 mg). At a given body size, vertebrates exhibit wide variation in absolute brain size, yet some basic patterns are evident. Within clades, smaller vertebrates have relatively large brains (Haller's Rule), and among smaller vertebrates in general, brain sizes may vary less than among larger vertebrates. Selection for maximizing brain size appears to be strong and may intensify at especially small body sizes. Do these same patterns hold for invertebrates? We measured body, head and brain volumes and masses of insects in several Orders, using a microvolumetric method that accommodates unfixed, freshly dissected specimens. We found a wide range in relative and absolute brain sizes: some insects invest in remarkably large brains; others clearly have followed alternative evolutionary paths that do not maximize investments in brain tissue. Brain sizes also varied widely relative to head size. Brain/head ratios differed by as much as 20-fold in volume and 60-fold in mass, and were significantly correlated with the brain/body ratio but not with head or body size. Thus, in addition to metabolic costs, investing in a bigger brain may entail competition with other tissues in heads for limited space, with important implications for evolutionary trade-offs. Our results suggest insect analogs to the proposed "brains vs. brawn" trade-off in hominid cranial evolution.

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Plenty of room at the bottom: brain miniaturization in ants

Prior studies show that vertebrate brain size scales to body size following a power law function, with significantly larger-than-expected brains in some lineages (e.g., Homo). Most animals are substantially smaller than vertebrates, but little is known of their brain allometry. Here we study the brain allometry of ants (N=70 species) encompassing about four orders of magnitude in body size and find that small ants (<0.9 mg body mass) have a significantly different brain allometry versus large ants. Brain allometry of a single polymorphic species (encompassing three orders of magnitude in body size), Atta colombica, follows a similar pattern with small individuals (<1.4 mg body mass) differing from large ants. The scaling exponent of small ants is comparable to that of higher mammals, and the smallest ants have brains that constitute ~15% of body mass, far exceeding that of all mammals. We also explore allometric patterns among the major subfamilies of ants and how scaling relationships differ among these groups. Broad comparative studies show how nervous systems can be miniaturized yet generate complex behavior systems such as those found in ants.

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Changes in hiding behaviour and the nervous system during the post-embryonic development of the locust Locusta migratoria.

Looming detection is crucial for animals to respond efficiently and escape from approaching stimuli. Using locusts as experimental models, extensive research has been done to characterise the neural circuits underlying this capacity. Until now, these studies have been conducted in adult locusts. Nonetheless, these animals avoid predation by birds throughout their lives and the preferred escape response displayed varies during development. The hiding response involves a positioning behaviour in which locusts sitting on branches or leaves tend to hide from an approaching stimulus by moving behind the object. The neural circuit underlying this behaviour is unknown. Because hiding responses are triggered by looming stimuli it is possible that the LGMD-DCMD system is involved. In this study we analysed the hiding responses displayed at different stages of the locust's life (first to fifth instars and adult). Although hiding responses were identified in all developmental stages, the proportion of animals displaying such responses changes dramatically in the different instars. Other parameters that are modified during development are the response latency (and thus the subtended angle of the looming object at the time of response), the response velocity and the angle of rotation performed during the response. Our results could be explained by a change in the behavioural strategy opted for by locusts at each instar or it could be correlated with an uneven maturation of the nervous system with some circuits developing earlier than others, or even by a change in visual acuity during development, particularly in the early instars. To asses the likelihood of these different explanations, we used Bodian's original reduced-silver method to reveal the neuroarchitecture of the optic lobes in each of the instars. An attempt was also made using Neurolucida to reconstruct the LGMD1 and LGMD2 neurons to reveal the presence and state of development of these neurons in different instars.

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Comparison of microglomerular structures in the mushroom-body calyx of neopteran insects

Mushroom bodies (MB) are prominent brain neuropils in the insect brain involved in higher order processing such as learning and memory, spatial orientation and sensory integration. The size and general morphology of the MBs is diverse across insects. Hymenoptera, for example honeybees, bumblebees or ants, possess particularly large and doubled MB calyces that are divided into subcompartments. In this study we comparatively investigated the microstructure of synaptic complexes (microglomeruli, MG) in the main sensory input regions of the MBs across various neopteran insect species. Pre- and postsynaptic compartments of MG were analyzed using anti-synapsin immunocytochemistry, f-actin-phalloidin labeling, high resolution confocal and electron microscopy. We focused on two major questions: 1) is there a common architecture of MG structures in the insect calyx, and 2) is there a higher level of complexity in the calyx of Hymenoptera compared to the calyx of other insects? Our results suggest that microglomeruli exist across all investigated neopteran insect species, but differences are found in the distribution of synapsin and f-actin in pre- and postsynaptic compartments. Synaptic quantification indicates that hymenopteran MBs contain the highest number and packing density of MG compared to all other insect orders investigated so far. We conclude that Hymenoptera have evolved high numbers of MG associated parallel synaptic microcircuits, which, most likely, lead to an increase in the computational capacities of the MB calyx. Supported by HFSP and SFB 554 (A8).

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Muscarinic activation regulates developmental and experience-dependent structural plasticity of honey bee Kenyon cells

The honey bee (Apis mellifera) brain is a model system for the study of experience-dependent neuronal plasticity. Young worker bees perform in-hive tasks while older bees exit the hive to forage. The Kenyon cell dendritic fields of 1 day old bees are more complex than their 7 day old sisters and bees with more foraging experience have larger arbors than age-matched, less experienced bees [1]. We hypothesize that foraging induced mushroom body growth is mediated via the same activity-dependent mechanisms seen during development. In the present study, we used pharmacologically manipulated cholinergic signaling in the brains of 1 day old and known age and experience forager bees. On the day of emergence or after accumulating 1 week of foraging experience, bees were caged in the laboratory and treated with pilocarpine (muscarinic agonist) or scopolamine (muscarinic antagonist) for one week. The Golgi method was used for detailed studies of Kenyon cell arborizations. Pilocarpine treatment prevented, but scopolamine treatment increased, the normal developmental pruning which occurs in young bees. Foragers also had increased dendritic complexity with pilocarpine treatment. These data suggest that the dendritic arbor of Kenyon cells is affected by the level of muscarinic activation. [1] Farris et al. J. Neuroscience 2001 21, 6396-6404

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Patterns of cell birth and cell death reflect variability in orientation behavior after treatment with ototoxic agents in developing Rana catesbeiana tadpoles

Administration of ototoxins to the inner ear of mammals destroys hair cells and produces irreversible hearing loss and vestibular disruption. Levels of active caspase-3, a marker for apoptosis, are correlated with severity of hair cell loss. In amphibians, inner ear and lateral line hair cells can spontaneously recover after damage. We examined the effects of ototoxin-induced damage and subsequent regeneration on active caspase-3 expression and lateral line/ vestibular functions in Rana catesbeiana tadpoles. Tadpoles were treated with different dosages of gentamicin solution (0, 100 µM, 500 µM) either by immersion or by direct injection into the otic capsule, thus differentially affecting lateral line and inner ear hair cells. After undergoing behavioral testing of balance, swimming, and detection of water flow, tadpoles were sacrificed and their brains processed for immunohistofluorescent labeling for caspase-3 and PCNA, a marker for proliferating cells. Apoptotic cells were also identified using hemotoxylin staining. Behavioral changes associated with gentamicin treatment included a disruption of balance, altered swimming, and an inability to detect water currents. These behaviors did not show recovery by 7 days after gentamicin exposure. Animals treated with gentamicin showed increased caspase-3 expression in the brainstem compared to non-treated controls. Additionally, animals allowed a 7 day recovery period showed lower caspase-3 label than those sacrificed immediately after treatment. These results suggest that damage to hair cells is reflected in cell death in appropriate target areas. Given that affected behavior was not recovered after 7 days, the results also imply ongoing changes in central target areas of the brain during the process of peripheral hair cell regeneration. [Supported by NSF GRFP, NIH and RI Space Grants]

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P 10

Upregulation of cell proliferation in the brainstem in early stages of nerve regeneration in adult Xenopus laevis

Frogs have the ability to regenerate cranial nerves after damage, but the central consequences of peripheral disconnection and reconnection have not been thoroughly investigated. We crushed the eighth cranial nerve of adult Xenopus laevis frogs unilaterally, with the contralateral nerve serving as an unoperated control. Animals survived up to 30 days post nerve crush, during which time their vestibular reflexes were tested. They were then sacrificed for anatomical verification of nerve damage and reinnervation of the medulla, and for evidence of new cell proliferation, as indexed by label of BrdU, in central targets of the nerve. Behavioral recovery remained incomplete up to 30 days survival, suggesting that Xenopus do not show considerable vestibular compensation. This lack of full recovery was correlated with incomplete reinnervation of the medulla. At both 7 and 30 days post nerve crush, there was considerable BrdU label, well over baseline levels, in both the ipsilateral and contralateral medulla. This bilateral upregulation suggests that commissural activity plays an important role in mediating central events during early periods of regeneration.

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Cavefishes as models for sensory adaptation.

Adaptation is the context in which behaviors and underlying nervous systems evolve. A quick change of environments poses a challenge for adaptation and extreme environments pose harsh. Therefore such environment provides valuable insights in to the evolutionary malleability of nervous systems. The permanent darkness present in caves imposes harsh sensory constraints that offer a distinct opportunity to examine how sensory modules not only become transformed, but also how they influence each other's changes. Troglodytic animals are known to have specialized sensory systems as outcomes of both constructive and regressive traits. Of all cave dwelling vertebrates, cavefishes are an especially suitable animal model for comparative studies because of their diverse phylogeny and world distribution. Moreover, once fish have colonized a cave they rarely re-enter or leave the cave so the duration of adaptation can be more easily determined and less complicated to describe. Species of cavefishes have independently colonized caves all over the world. At a first glance all have lost eyesight but it is unclear what modalities if any have become hypertrophied as a response and rules are yet to be established. Here we examined two cavefishes Ogilbia pearsei (Yucatan, Mexico) and Astroblepus pholeter (Napo, Ecuador).

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Auditory Systems

588 **P 12**

Echolocation strategy of free-ranging FM bats, Pipistrellus abramus, during target search, revealed by a microphone-array system

Aerial-feeding insectivorous bats capture hundreds of small airborne insects a night. Here, we measured sonar emissions and 3-D flight paths of wild Pipistrellus abramus during foraging insect preys by using a custom made microphone-array system consisted of four units. Each unit was consisted of four 1/8-inch omni-directional condenser microphones arranged in a symmetrical Y-shaped formation. Two units facing same direction were installed on one side of a riverbank and the other two units rotated opposite direction were set up on the opposite bank, facing the area where the bats have been observed. The 3-D flight paths of foraging bats were continuously tracked while the bats captured multiple insects within 10-15 squared meters and 4-9 meters high above the river surface for almost 1 minute. The flight areas got larger three-dimensionally and showed simpler circular pattern when the capture repetition rate was low (1 capture in 22 s) compared to the condition when the capture repetition rate was high (22 captures in 49 s). In the search phase, the bats emitted quasi constant-frequency (CF) pulses, and the CF duration increased as a function of flight altitude. The interpulse interval (IPI) was relatively constant at around 90-110 ms during the search phase. The search range estimated from the IPI of 90-110 ms was 5-9 meters which approximately corresponded to the flight altitude of the bats. Interestingly, P. abramus prolonged IPI occasionally longer than 150 ms (long IPI), which was observed frequently in the case of low capture repetition rate. The estimated search range from the long IPI was 15-24 meters which approximately corresponded to the area where the bats have been intensively observed. This finding suggests that the bats may frequently expand their search range by prolonging IPI in order to find new foraging area when the insect density is low, which might be an effective search strategy used by bats. [Research supported by JSPS and ONR grant]

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Onset evoked inhibition from excitatory stimuli in duration-tuned neurons

Auditory neurons selective for stimulus duration, referred to as duration-tuned neurons (DTNs), are first observed in the inferior colliculus (IC) of both echolocating and non-echolocating mammals. Although the behavioural significance of duration-tuning is currently unknown, DTNs provide a mechanism for temporal processing. Bat DTNs exhibit especially high temporal selectivity (on the order of milliseconds) that may function in processing of echolocation calls. Duration-tuned neurons are created from a combination of excitatory and inhibitory synaptic inputs that are offset in time [1,2]. Non-excitatory tone durations have previously been shown to evoke long-lasting (i.e. persistent) inhibition in DTNs [1]. Here we examined the time course of spike suppression (inhibition) evoked by pairs of excitatory tones in both DTNs (presented at best duration) and in non-duration selective IC neurons. Using paired tone stimulation and single unit extracellular recording in the IC of the big brown bat (Eptesicus fuscus) [1], we stimulated cells with pairs of characteristic frequency tone pulses that differed in their interstimulus interval to quantify the strength and time course of inhibition acting on DTNs and non-DTNs. We observed a decrease in the number of spikes evoked by the second tone in the pulse pair as the interstimulus interval decreased. We found that spike suppression lasted longer for DTNs than for non-DTNs. In addition to supporting previous observations that DTNs receive persistent inhibition, this also suggests that DTNs receive longer lasting inhibition compared to other types of IC neurons. This study helps to elucidate the mechanisms underlying duration selectivity in the auditory midbrain of mammals. Research supported by NSERC. [1] Faure PA, Fremouw T, Casseday JH, Covey E (2003). J Neurosci 23:3052–3065 [2] Aubie B, Becker S, Faure PA (2009). J Neurosci 29:9255–9270

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Local neuronal networks within the cochlear nucleus of the echolocating big brown bat, Eptesicus fuscus.

Echolocating big brown bats (Eptesicus fuscus) can detect changes in echo delay of a microsecond or less. The bat's cochlear nucleus (CN) is a likely site for neuronal mechanisms supporting this unique temporal hyperacuity. The bat's CN displays high levels of expression of connexin 36 (Cx36), a structural protein in neuronally-expressed gap junctions. In contrast, Cx36 is not expressed in the CN of wild-type mice, non-echolocating mammals with a similar ultrasonic auditory range. To determine if Cx36 indicates the presence of functional electrical synapses, we made focal injections of neurobiotin, a tracer molecule capable of passing through gap junctions, into subnuclei of the bat's CN as well as in the auditory nerve (nVIII). There was no gap junction mediated travel between the nVIII and the CN. Injections in the anteroventral (AVCN) and posteroventral (PVCN) CN revealed axonal projections to the superior olive and inferior colliculus. Injections in the ventral region of the AVCN labeled a population of large multipolar cells on the margins of the AVCN surrounding the entering axons of the nVIII with extensive interconnectivity but limited ascending projections. These cells may be interconnected via gap junctions and thus constitute an electrically-coupled local network that forms a spectrotemporal gateway for supporting the temporal hyperacuity necessary for biosonar.

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It's About Time: How Input Timing is Used and Not Used to Create Emergent Properties in the Auditory System

Frequency Modulations (FMs) are major components of bat echolocation and communication calls, and are common in communication across species, including humans. Previous work has shown that neuronal response selectivity for FM direction often predicts response selectivity to conspecific vocalizations. For over 35 years, the major hypothesis for FM directionality invokes a mechanism with an honored tradition in sensory neurobiology: the relative timing between excitation and inhibition. The timing disparity is created by the asymmetrical locations of excitatory tuning and inhibitory sidebands. Inhibitory sidebands tuned to frequencies lower than excitation creates downward selectivity. FMs sweeping downward activate excitation first; the initial excitation is unopposed by inhibition, evoking discharges. Upward FMs evoke inhibition that either leads or is coincident with the excitation, suppressing discharges. Here we evaluated FM directionality with in vivo whole-cell recordings from the inferior colliculus (IC) of awake bats. From the recordings, we derived synaptic conductance waveforms evoked by down- and upward FMs that swept identical frequency ranges. In a model, the derived conductances accurately predicted sound-evoked postsynaptic potentials (PSPs). When we tested the effects of shifting inhibition relative to excitation in the model, we found that EPSP amplitudes were remarkably insensitive to small latency shifts (often < 1 mV/ ms). Importantly, the effect of shifting inhibition depended strongly on initial differences in the latencies and shapes (temporal envelopes) of excitation and inhibition. Finally, when the PSPs peaked close to spike threshold, even small latency shifts could cause some cells to fire more strongly to a particular FM direction, and thus change its directionality. We found that timing is more than latency differences between excitation and inhibition, and that directionality depends on a complex interaction between conductance temporal envelopes, size, and spike threshold.

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Motion Detection as a Neural Mechanism for Encoding Natural Communication Signals in the Bat Inferior Colliculus

Natural sound, such as conspecific vocalizations and human speech, represents an important part of the sensory signals animals and humans encounter in their daily lives. Sensory neurons in the auditory system must have evolved to process sounds that are vital for social communication, foraging, mating and therefore survival. Previous studies have shown that response selectivity for conspecific vocalizations can be observed as early as the inferior colliculus (IC) in the auditory midbrain. Receiving a convergence of excitatory and inhibitory inputs from the brainstem, it is not surprising that emergent response properties like feature selectivity can arise in the IC. In this study, we recorded the extracellular response of bat IC neurons to a large repertoire of conspecific communication signals. This allowed us to map the overall receptive field of each neuron by deriving a set of linear filters that represent the relevant stimulus features each neuron is tuned for, together with a static nonlinearity that maps the output of each filter to the firing rate of the neuron. Most neurons in the bat IC were tuned for stimulus features that were inseparable and tilted in the frequency-time axis thus allowing them to be selective for the direction and velocity of frequency-modulated (FM) signals. In about half of these neurons, FM selectivity was achieved by having two linear filters that were both tuned for the preferred FM direction with a phase shift and a symmetric nonlinearity. Others had a second filter that was tuned for the non-preferred direction and suppressed the response of the neuron. The tuning of IC neurons for specific FM direction and velocities matched the distribution of upward and downward FM velocities found in the conspecific vocalizations of the bat. This close quantitative correspondence among features of sensory signals and neural tuning suggests that motion detection is the mechanism in which IC neurons encode features of conspecific communication signals.

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P 17

Frequency tuning and latency organization of inferior collicular neurons in Japanese house bat, Pipistrellus abramus

Japanese house bat, Pipistrellus abramus, emits quasi-constant frequency pulses during search, which extend the end frequency portion of the downward frequency-modulated sweep (terminal frequency; TF) as long as 10 ms. If the narrowed frequency range is important for detecting a small frequency change caused by insect fluttering, the bats may need much finer frequency resolution at the TF than other frequency ranges. To test this hypothesis, the distribution of the best frequencies (BFs) in the inferior colliculus (IC) was electrophysiologically measured. The TF of the echolocation pulse was 41.44 kHz on average. The frequency range of 35–45 kHz was overrepresented in the IC (n = 50/105; 48%), and a faint second peak was also seen at 75–85 kHz (the second harmonic of the TF) in the BF distribution. The BF increased as a function of recording depth along the dorso-ventral axis, except for the BFs of 35–45 and 75–85 kHz, which were found at a wide range of depths. The response latency ranged between 3.7 and 23.2 ms for the BFs of 35–45 kHz, and the maximum target range was estimated to be 3.3 m from the delay line observed in the IC. These electrophysiological measures suggest the importance of a target distance within approximately 3 m, which agrees with behavioral measures during foraging in this species. [Research partly supported by ONR grant #00014-07-1-0858].

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Neurons in the inferior colliculus of the mustached bat are tuned to both call duration and call-echo delay

The present study examines duration and delay tuning in neurons of the inferior colliculus of the mustached bat Pteronotus parnellii. Studies on duration- and delay-tuning have been conducted independently in FM and CF-FM bats, in which similar operational time ranges (1 - 20 ms) have been found. Furthermore, for both types of time tuning the underlying mechanisms have been modeled using similar inhibitory and excitatory neuronal inputs with specifics temporal relations. It is thus unknown whether the same neuron could selectively respond to certain durations and delays and whether the same mechanisms are involved. Consistent with results from other mammals, duration-tuned neurons in the IC of the mustached bat fall into three main types: short-, band- and long-pass. The range of best durations in the inferior colliculus of this species encompass the duration of their echolocation signals (up to 27 ms). In most of the duration tuned neurons, response areas as a function of stimulus duration and intensity took either V- or U shape, with duration tuning retained across the range of intensities tested. Duration tuning was affected by changes in sound pressure level in only six neurons, in which a general tendency to decrease best duration with increasing intensity was observed. These neurons may "track" the decreasing duration of the echo during a pursue sequence where echo duration is decreasing and intensity is increasing. Delay tuning was also examined with a combination of the first-harmonic frequency modulated sweep in the emitted pulse and the higher harmonic frequency-modulated component of the returning echoes. From 50 neurons, 28 were tuned both to duration and call-echo delay. In 15 duration-tuned neurons best duration and best delay coincide, whereas in 13 duration-tuned neurons best duration differs from best delay. We propose that these findings can be explained by adjusting the timing of the delayed excitatory inputs and their coincidence or not with inhibitory rebounds.

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P 19

Pulse-echo interval selectivity in models of duration-tuned neural circuits predict a potential role in echolocation

Duration-tuned neurons (DTNs) are found in the auditory midbrain (inferior colliculus) and are selective for specific ranges of stimulus durations. While not exclusive to echolocating species, bat DTNs tend to have high temporal resolution in comparison to other mammals. In this study, we employed a computational model of duration tuning to investigate whether bat DTNs may play a role in echolocation. Our model has previously been shown to replicate a variety of *in vivo* DTN response properties such as spike counts, first-spike latencies, level tolerance, and the effects of blocking synaptic inhibition with neuropharmacological antagonists [1].

To investigate if DTNs are sensitive to pulse-echo stimuli as seen in echolocation, we presented the model of duration tuning with a high intensity 1 ms pulse followed by a low intensity 1 ms echo separated by different temporal intervals. Interestingly, for short interstimulus intervals (*in vivo* comparisons of the model"s prediction.

[1] Aubie, Becker & Faure (2009) J Neuroscience 29:9255-9270

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416 **P 20**

Development of Frequency Modulated Vocalizations in Big Brown Bat Pups

Developing bat pups produce distinct vocalizations referred to as isolation calls (I-calls) that serve to attract the bat's mother, and mothers use auditory and olfactory cues to reunite with their offspring. Because I-calls are unique to individual pups, vocalizations are assumed to be crucial for the reunion process. How individual pups shift their vocalizations from I-calls to frequency modulated (FM) sweeps during development remains unclear. By recording individual big brown bat pups from the day of birth to twenty-five days of age we have observed vocal behavioural changes as well as acoustic (temporal and spectral) changes in pup calls. The temporal characteristics examined were call duration and intercall interval; the spectral characteristics were the minimum, maximum and peak spectral frequency. I-calls are produced only until a certain point in development, after which the pups change from emitting long-duration simple tonal calls to downward FM signals and eventually to short-duration biosonar vocalizations used in echolocation. We discovered additional spectral changes in the harmonic structure of pup calls as the bats age. Specifically, the number and bandwidth (richness) of harmonic elements decreased with age. We also recorded pup vocalizations after set lengths (15 min. to 2 hours) of prolonged separation from their mothers to determine if extended isolation alters the type, number or acoustic structure of calls. Increased physiological stress and reduced body temperature resulting from prolonged isolation may cause pups to alter the content of their I-calls, indicating an increased need for maternal assistance. Alternatively, pups may reduce calling to conserve energy during extended isolation. By documenting the acoustic behaviour of developing pups in different auditory environments, we can provide groundwork for further research on FM vocalization development. Graduate fellowship and research support by NSERC.

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New degrees of freedom in fruit bat echolocation

Bats from the genus Rousettus use a unique strategy of echolocation. Instead of emitting tonal laryngeal calls like most bats they emit wide-band short (~100 microseconds) Dirac-like lingual clicks. In contradiction to the previous belief, recent experimental and theoretical results suggest that click based echolocation used by the Rousettus genus cannot be disregarded as primitive. Here we shall present the echolocation behavior of six Egyptian fruit bats (Rousettus aegyptiacus) that were trained to detect, localize and land on a target in acoustical scenes with different levels of complexity. Using 2 video cameras and a 20-micerophone array we tracked the position of the bats and estimated their beam shapes and beam directions while performing the tasks. The bats' behavior in the simple acoustical scene (only target in the room) revealed an interesting pattern [1]: Contradicting previous notion, the bats did not center their sonar beam on the target, but instead pointed it off-axis, accurately directing the maximum-slope of the beam onto the target. Information-theoretic calculations showed that using this maximum-slope strategy is optimal for localizing the target – at the cost of detection (SNR). We further proposed and showed that there exists a tradeoff between detection (optimized at stimulus-peak) and localization (optimized at maximum-slope) and suggested that it is fundamental to spatial localization and tracking accomplished through various sensory systems. The comparison of the bats' behavior in scenes with different levels of complexity (more or less than one target in the room) shows that they can modulate the area covered by their bio-sonar system by altering both the direction of the beam as well as its width. These results suggest a new echolocation degree of freedom possessed by these bats. 1) Y. Yovel, B. Falk, C. F. Moss, N. Ulanovsky, (2010) Optimal localization by pointing off-axis. Science 327, 701-704, 28.1; 1/42;

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257 **P 22**

Hetero-harmonic echo-delay selectivity in the auditory cortex of the FM bat Pteronotus quadridens

Echolocating bats crucially depend on a precise cortical computation of echo-delay to determine target range. There are two strategies known to explain how this task could be solved. (i) Bats emitting combinations of constant frequency and downward frequency modulated calls (CF-FM bats) compute the time interval between the emission of a lower frequency harmonic in the call and the incoming echoes from a higher frequency harmonic (hetero-harmonic). (ii) On the other hand, bats emitting purely downward frequency modulated calls (FM bats) compute the echo-delays by using call-echo pairs of similar frequencies (homo-harmonic). In contrast to other FM species, in the auditory cortex of the FM bat Pteronotus quadridens the echo-delays are computed hetero-harmonically. In this species, the 72% of the neurons are selective to echo-delays ranging between 1-18 ms, with most of them responding only to combinations of the first harmonic in the calls (FM1) and a delayed higher harmonic (FMx). Echo-delay selective neurons of P. quadridens have both delay tuned responses in which the best delay does not depend on the echo-amplitude, and tracking responses in which the best delay decreases as increasing the amplitude of the echo. The characteristics of echo-delay selectivity in the cortex of P. quadridens closely match those observed in its congeneric Pteronotus parnellii (a CF-FM bat), suggesting that there is an important influence of phylogeny in the determination of ranging strategies.

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Amygdalar auditory responses to social vocalizations in big brown bats

We examined how social vocalizations defined by emotional state evoke auditory responses in the amygdala. We recorded and analyzed 17 types of social calls from big brown bats (Eptesicus fuscus), and examined how they varied with behavioral context and emotional intensity of the caller. During aggressive encounters, bats commonly emit a broadband, downward FM syllable that is rapidly repeated in simple phrases. With increasing levels of aggression, the repetition rate and number of syllables per phrase increased, and additional syllable types were more likely to occur at the end of the phrase. In single unit recordings from the lateral amygdala of awake bats, we obtained auditory responses to a diverse set of social vocalizations and simple acoustic stimuli. To characterize responsiveness to vocal calls, we examined whether spike discharge rate was significantly above background activity. Based on this criterion, amygdalar neurons responded to a broad variety of social vocalizations and simple acoustic stimuli. Most neurons (95%, 39/41) displayed little selectivity, responding on average to 57% of the 22 social calls tested. However, this measure did not capture differences in response magnitude and duration. Average response duration was 38.5 ± 38.9 ms (range: 2.2-252.3 ms). In 43% of responses, response duration was prolonged, exceeding stimulus duration. We therefore developed a response duration preference index (RDPI). Units were more selective, responding on average to only 25% of the social calls tested. When grouped according to the behavioral context, aggressive vocalizations were more likely to elicit a prolonged discharge than appeasement vocalizations (27% and 16%, respectively; $p<0.03$). These results suggest that bat amygdalar neurons respond to a broad range of acoustic stimuli, but that prolonged responses may be critical for amygdalar representations of a stimulus related to plasticity, modulation of sensory processing, and emotional expression.

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Perception of shape and rejection of clutter are reciprocal processes in FM bat sonar

Echolocating big brown bats (Eptesicus fuscus) exploit the reliability of lowpass filtering for echoes that arrive from off-axis or far away to defocus their biosonar images for clutter while retaining sharply-focused images for objects located immediately to the front. During echolocation, bats render echoes into images that depict each object's distance from the delay of echoes and its shape from echo spectra. When tests with echoes whose amplitudes and spectra mimic reflections from a target located immediately to the front, bats perceive images depicting both target range and target shape with good acuity. When tested with echoes that are lowpass filtered to mimic reflections from off-axis clutter, the bat's images depict target range and shape with very poor definition. In effect, the images of clutter from the surroundings are profoundly defocused, while images of any objects located to the front are in sharp focus. Defocusing of echo-delay images goes hand-in-hand with rejection of clutter. When images of lowpass echoes are defocused in delay, these echoes do not cause masking. When images of flat-spectrum echoes are in focus, masking does occur. In that they are reciprocally related, acute perception of targets and rejection of clutter are two sides of the same perceptual coin. These two effects are coupled by the transformation of echo spectral notches into estimates of glint separations, part of the SCAT process. Lowpass-filtered echoes have weaker 2nd harmonic FM2 sweeps than FM1 sweeps. Amplitude-latency trading then causes loss of coherence in the bat's neural representations of the harmonics, which activates spectral transformations that defocus, or blur, the bat's delay images of clutter.

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Characteristics of echolocation pulse and flight trajectory in FM bats (Pipistrellus abramus) in the cluttered environment by chain obstacles

It is said that terminal frequency (TF) of echolocation pulses in Japanese FM bats (Pipistrellus abramus) was temporally extended when they were searching insects. TF is one of the most important characteristics of echolocation pulses. TF of echolocation pulses in FM bats during flight in cluttered environment was investigated by us. Chain-array obstacles were arranged in a flight chamber. Multiple reverberant echoes (echo stream) were returned from each chain. Echolocation pulses of the bats were recorded with a telemetry microphone (Telemike) mounted on the head so that changes of the terminal frequency (TF) could be precisely determined. However, sensitivity of a Telemike isn□ft enough to analyze echo stream. A fixed ultrasonic microphone that is more sensitive than Telemike, successfully recorded echo streams from the chain obstacles. The bats emitted pulses in pairs (strobe groups) with short (20-40 ms) interpulse intervals (IPIs) while the bats were heading to the chains. We regard TF shift frequency between each succeeding pulses. The maximum *ƒ¢*F was approximately 5 kHz while the bats were flying toward the chains, although *ƒ¢*F was much less (~2 kHz) changed while the bats were flying away from the chain obstacles. Echo stream duration (ESD) was around 40 ms when the bats were flying toward the chain obstacles. The large amount of *ƒ¢*F was observed when IPI was less than 40 ms. On the other hand, when IPI was longer than 40 ms, *ƒ¢*F was around 2 kHz. This indicates that the bats shifted TF much larger when echo streams were overlapped in time within strobe group. If the bats emit a strobe group with the same TF during flight in clutter, they may not be able to segregate pulses in each echo stream from different obstacles. Our findings suggest that the bats avoid interference between their own echo streams by shifting TF of pulse. [Research supported by JSPS and ONR grant]

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FM echolocating bats shift frequency of sonar sounds to avoid acoustic ambiguity in clutter

Echolocating bats determine the distance to objects by using the delay of echoes from each emitted pulse. When pulses are emitted rapidly in cluttered surroundings, echo streams from successive pulses overlap, which causes acoustic ambiguity in matching echoes to corresponding emitted pulses. To investigate how bats react such pulse-echo ambiguity in clutter, the big brown bats (Eptesicus fuscus) were trained to fly in dense clutter-producing obstacles constructed by multiple rows of vertically hanging chains. The bat"s sonar pulses were recorded by a custom-made telemetry microphone (Telemike) mounted on the bat"s back so that acoustical characteristics of sonar pulses could be monitored without artifacts caused by the bat"s movements with respect to the recording microphone. A series of echoes from multiple rows of chains were recorded with stationary ultrasonic microphone placed on the floor. When the bats flew in area surrounded by the chain array, chain echoes arrived over a span of time ranging from 10 to 50 ms following each emitted pulse. The bats emitted pulses in pairs (strobe groups) at short (20-40 ms) interpulse intervals (IPIs) alternating with longer IPIs (>50 ms). The bats sometimes emitted their second pulse of strobe group while the long reverberant chain echoes from the first pulse were still being present. We found that when their own echo streams from successive pulses in strobe group overlapped in time (pulse-echo ambiguity), the bats slightly shifted frequencies of the first pulse in each strobe group upward and the second sound downward by 3-6 kHz. When this pulse-echo ambiguity ceased, frequency shifts ceased also. The bats appear to recognize echoes as "belonging" to specific pulses based on small frequency differences in their FM sweeps. This result suggests self jamming avoidance of FM echolocating bats; shifting the frequency range of outgoing sonar pulses to segregate overlapped echo images. [Work supported by ONR]

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A stylohyal-tympanic connection signals laryngeal echolocation in bats

Echolocation is an active form of perception where animals emit sounds and then listen to the reflected echoes to form images of their environment in their brain. For this process to work outgoing sounds must be represented at a neuronal level for future comparison with returning echoes. The mechanism effecting this neuronal representation is presently unknown. The ability to echolocate has evolved at least four times in mammals (bats, whales, shrews, tenrecs) and twice in birds (oilbirds and cave swiftlets). Although echolocation is usually associated with bats, it is not characteristic of them. Most echolocating bats emit sounds from the larynx, but within one family (Pteropodidae) of mainly non-echolocating bats a few species emit broadband unstructured sounds by clicking their tongue. Using anatomical data obtained from micro-computed tomography scans of 26 species (n = 35 fluid-preserved bats), we found that proximal articulation of the stylohyal bone (part of the mammalian hyoid apparatus) with the tympanic bone always distinguishes laryngeally-echolocating bats from both non-echolocating and tongue-clicking pteropodid bats. The stylohyal bone functions in breathing, swallowing and phonation; the tympanic bone surrounds and supports the tympanic membrane. In many high duty cycle bats the stylohyal bone was fused to the tympanic bone. A previous report on the stylohyal bone in the oldest known fossil bat (Onychonycteris finneyi) suggested that it did not echolocate; however, we speculate that O. finneyi may have used laryngeal echolocation because its stylohyals may have articulated with its tympanics. Coupling the larynx to the ear via a stylohyal-tympanic connection could serve multiple functions in hearing and echolocation, and provides an independent anatomical character to distinguish laryngeally-echolocating bats from all other bats. Our discovery reopens basic questions about the timing and the origin of flight and echolocation in the early evolution of bats.

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Frequency and intensity dependent properties of stimulus-specific adaptation in the inferior colliculus

The ability to detect unexpected sounds from the environment is an important function of the auditory system as they may need an immediate adaptive response. Recent studies using an oddball paradigm have found a decreased response to a repetitive stimulus (standard) but an increased response to rare and less frequent sounds (deviant) both in the auditory cortex (Ulanovsky et al., 2003) and subcortically (Pérez-González et al., 2005; Malmierca et al., 2009; Antunes et al., 2010). This phenomenon, known as stimulus-specific adaptation (SSA), has been suggested to serve for change detection and as a single neuron correlate of certain evoked potentials. Currently is not fully understood how SSA varies within the responsive field of a single neuron, i.e. it is unclear whether SSA is a unique property of the neuron or a feature that is frequency and/or intensity dependent. In the present experiments we used the weighted SSA index (WSI; Ulanovsky et al., 2003) to quantify and compare the degree of SSA under different stimulation conditions. This descriptive statistic analyzes the neuronal adaptation to a determined pair of frequencies in an oddball paradigm. We calculated the WSI at different levels in each individual neuron, in order to map the neuronal WSI within the receptive field. Our preliminary data show a higher adaptation to frequency at low intensity sounds, i.e. close to threshold. This finding suggests a stronger adaptation to strange, discrete sounds from the acoustic scene and puts forward the idea that SSA varies as a function of sound intensity. We speculate that the functional mechanisms that mediate SSA may have an elaborated control and play a more important role in change and novelty detection that previously estimated. Acknowledgements: Financial support was provided by the Spanish MEC (BFU2009-07286), EU (EUI2009-04083) and JCYL-UE (GR221) to MSM. DDD held a fellowship from "La Caixa" Foundation.

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Nicotinic receptors in the rat brainstem are involved in prepulse inhibition of startle - a measure of sensory gating

The startle response is a physical reaction to a sudden and intense sensory stimulus which can be attenuated by a non-startling sensory stimulus (prepulse) presented 5-1000ms before the startle-evoking stimulus. This prepulse inhibition (PPI) is a measure of sensory gating that is necessary to filter-out sensory stimulation and avoid sensory information overflow. In the rat, giant neurons in the caudal pontine reticular nucleus (PnC) mediate startle responses and they receive modulatory input from the pedunculopontine tegmental nucleus (PPTg), which has been proposed to mediate prepulse inhibition and contains a huge number of cholinergic projections. Electrophysiological studies from Bosch and Schmid (2006, 2008) have confirmed cholinergic PnC inhibition by PPTg fibers, however, the application of a muscarinic blocker only partly reverses cholinergic inhibition, leaving a possible role for brainstem nicotine acetylcholine receptors (nAChRs) in PPI. We tested this by systemic and local PnC injections of nicotine and nicotinic antagonists in vivo. Systemic administration of nicotine (1mg/kg, SC) significantly enhanced PPI ($p<0.01$) while intra-PnC injections of nicotine (10mM, 1.0 uL) severely disrupted PPI ($p<0.001$). Intra-PnC injections of TMPH, but not MLA, which block the $\alpha4\beta2$ and the $\alpha7$ nAChRs, respectively, also disrupted PPI ($p<0.01$). To summarize, this research is the first to identify brainstem nAChRs as a mediator PPI of the acoustic startle response. The $\alpha4\beta2$ nAChR subtype appears to be involved in this mediation of PPI. Since the systemic injections of nicotine enhanced PPI, but local injections of nicotine into the PnC had the opposite effect, we propose that the systemic effect of nicotine on PPI is not mediated by brainstem nicotine receptors, but rather by higher brain areas that have been shown to modulate PPI.

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P 30

GABA modulates stimulus-specific adaptation in the inferior colliculus of the rat

Neural responses in the auditory nervous system depend not only on the current stimulus but also on the history of past stimulation. One form of this history-dependence is stimulus-specific adaptation (SSA), i.e., the reduction in the responses to a common sound relative to the same sound when rare, which has been described at cortical (Ulanovsky et al., 2003) and subcortical levels (Pérez-González et al., 2005; Malmierca et al., 2009; Antunes et al., 2010). At present the functional mechanism that generates SSA is unknown. We have previously demonstrated that the type of SSA seen in the inferior colliculus (IC; Malmierca et al., 2009) is very similar to that of the auditory cortex (Ulanovsky et al., 2003, 2004). This finding raises the question of whether SSA is created at the midbrain or in contrast it is inherited from the cortex. Here we investigated the role of locally GABA-mediated inhibition in the generation of SSA in the IC. Using an oddball paradigm, we recorded single units in the IC before, during and after microiontophoretic application of gabazine (GBZ), a GABA-A receptor antagonist, to test whether GABAergic inhibition is shaping SSA subcortically. The results show that GBZ usually produces an increment of the neuronal firing rate and a shortening of first spike latencies (FSL). These changes depended on the probability of the stimulus presented. The relative increment in firing rate during application of GBZ was larger in the response to the standard stimuli. The FSL in response to deviant stimuli was generally shorter than in response to standards, and it was hardly affected by GBZ. In contrast, FSL in response to standards was significantly reduced by GBZ. Our findings indicate that GABA has a differential effect depending on the probability of the stimuli, and thus has the potential to modulate SSA locally in the IC. Acknowledgements: Financial support was provided by the Spanish MEC (BFU2009-07286), EU (EUI2009-04083) and JCYL-UE (GR221) to MSM.

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A newly discovered superoantero-orbital sinus connecting to the interaural canal may play a role in zebra finch hearing

The middle ears of birds are acoustically connected through an air-filled pathway, the interaural canal (IAC), which allows sound to propagate internally from one ear to the other and considerably enhance the cues for directional hearing. The enhancement of the directional cues depends on the amplitude gain and time delay through IAC. Theoretically, different combinations of frequency dependent gains and delays produce very different directionalities of the ears but it is still uncertain how gain and delay can be shaped by evolution. We have discovered that a large forehead sinus superoanterior to the orbits (superoantero-orbital sinus, SAOS) connects to the IAC via a tube inferomedial to the orbits (IMT). SAOS has a very complex shape with connections through an arc over the eye to the bullae of each ear and also to two smaller lateral sinuses in front of each eye. The contribution of this structure to auditory sensitivity has not been studied previously. We hypothesized that the extra air volume could form a resonator influencing the gain and delay of IAC. We tested the hypothesis by measuring sound transmission through IAC of zebra finches before and after filling the SAOS with a silicone impression medium. Six out of seven individuals showed an effect of filling the SAOS or the IMT on directionality and overall auditory sensitivity. The SAOS therefore does seem to have an effect on gain and delay in the interaural canal as well as on auditory sensitivity. The effect, however, was complex and either significantly changed the gain by up to 10 dB in the frequency range below about 2-3 kHz and/or in a narrow band around 4-6 kHz. Although we verified the fillings by post-experimental dissection, it was difficult to determine exactly to what degree the different components were filled. Thus differential filling and/or blockage of the complex system may be the reason for complexity of the results. Future experiments aim for better control of the cavity filling.

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Peripheral auditory processing changes seasonally in Gambel"s white-crowned sparrows

Songbirds produce songs in a variety of reproductive and social contexts and are therefore excellent models to study the development and maintenance of vocal communication. Like many other songbirds, Gambel's white-crowned sparrow (GWCS, Zonotrichia leucrophrys gambelii), breeds seasonally and exhibits dramatic seasonal differences in song production. The sex steroid hormones, testosterone and estrogen, mediate these seasonal changes in behavior and the morphological and physiological changes of the neural circuitry underlying song output. Less understood, however, is the effect of seasons and hormones on songbird auditory processing. To address this issue, we manipulated photoperiod and hormone levels of wild-caught GWCS in the laboratory to simulate natural breeding and non-breeding conditions. Peripheral auditory function was assessed by measuring the auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs) of males and females in both conditions. Birds exposed to breeding-like conditions demonstrated elevated ABR thresholds and prolonged peak latencies compared with birds housed under non-breeding-like conditions. Studies of other aspects of auditory processing, including otoacoustic emissions and ABR temporal and spectral tuning, revealed no effect of breeding condition. These results suggest that seasons and hormones modulate sensory processing at the level of the auditory nerve in wild songbirds.

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Neurophonic maps of ITD in the avian nucleus laminaris

Axons from the nucleus magnocellularis (NM) and their targets in nucleus laminaris (NL) form the circuit responsible for encoding interaural time differences (ITD). In barn owls, NL receives bilateral inputs from NM such that axons from the ipsilateral NM enter NL dorsally, while contralateral axons enter from the ventral side. This interdigitating projection into NL is tonotopic, and the afferents and their synapses on NL neurons generate a tone induced evoked potential, or "neurophonic," that also varies systematically with position in NL. From dorsal to ventral within the nucleus, the best ITD of the neurophonic shifts from far contralateral space to best ITDs near 0µs. Earlier recordings of the neurophonic showed that in NL, iso-delay contours ran parallel to the dorsal and ventral borders of NL (Sullivan and Konishi, 1986). This map is orthogonal to that seen in chicken NL, where a single map of ITD runs from around 0µs ITD medially to far contralateral space laterally (Koppl and Carr, 2008; Joseph and Hyson, 1993). Yet the trajectories of the NM axons are similar in owl and chicken (Young and Rubel, 1983). We therefore developed analytical techniques to measure delays in barn owl and chicken. Clicks were used to measure conduction time, while lesioned mark the 0µs iso-delay contour in multiple penetrations along an iso-frequency slab. In general, iso-delay contours were parallel to the dorsal and ventral borders of NL. Along an iso-frequency and iso-delay contour, latency increased systematically from medial to lateral in NL, for both ipsi-and contralateral axons. The 0µs iso-delay contour was close to the ventral border of NL, except in medial NL, where 0µs was found more dorsally. In chickens, along an iso-frequency contour, latency increased systematically from medial to lateral in NL, with larger increases in latency for contralateral axons than for ipsilateral axons.

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P 34

Underwater hearing in the red-eared slider turtle, Trachemys scripta elegans

The inner-ear of the red-eared slider has been studied extensively, primarily as an in vitro model because of the tolerance of turtle tissue to low oxygen tension. By comparison, very few studies have focused specifically on the sensitivity of this turtle ear in vivo. Here we report on an in-vivo study using auditory brainstem responses (ABR) and laser vibrometry in lightly anesthetized turtles. ABRs were measured using three subcutaneous electrodes. We used four modes of stimulation: 1) a closed coupler sealed over the eardrum; 2) underwater sound 3) dorsoventral vibration and 4) direct motion of the tympanic disk. Laser vibrometry was used to measure eardrum vibration velocities in the submerged animal. The tympanic disk is framed by a delicate membrane and very compliant. The head of the middle ear bone is firmly attached to the disk. Behind the disk is a large, air-filled cavity (volume about 0.95 ml, corresponding to a resonance frequency of approximately 500 Hz underwater). The ABR audiogram is V-shaped with best sensitivity to airborne sound (50 dB SPL) at 300-500 Hz. Although the turtle ear looks like a normal tympanic ear, audiograms before and after removing the skin covering the cartilaginous tympanic disk were unchanged, indicating that the tympanic disk, not the skin, is the key sound-receiving structure. Thresholds for direct vibration of the disk are approximately 2 µm/s at the most sensitive frequencies. A comparison of the audiograms show that the ear is usually 10dB less sensitive to underwater sound pressures than to airborne sound. Comparisons in terms of sound intensity, however, show that thresholds in water are lower than in air, indicating that the ear is specialized for underwater hearing. Given the structure of the tympanic disk and the large middle ear cavity, we hypothesize that the sound-induced particle motion of the air in the middle ear cavity drives the tympanic disk underwater.

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Unusual high-frequency hearing and vocalizations in pygopod geckos.

Hearing and vocalizations of amniote vertebrates are generally thought to differ systematically in their frequency ranges: the upper frequency limit had been shown to be lowest in chelonians and increasingly higher in crocodilians, lizards, birds and mammals. Pygopods are a legless subfamily of geckos endemic to Australasia and possess like them a highly specialized hearing organ. We report data from different pygopod species from the Pilbara region of Western Australia including four species of the genus Delma studied using recordings of auditory-nerve compound action potentials (CAP) measured in a mobile laboratory under remote field conditions. The upper frequency limits of the unspecialized genera Pygopus and Lialis were not exceptional, resembling those of other geckos (6-8 kHz). Hearing limits and vocalization energy of the Delma species, however, extended to frequencies far above those reported for any other lizard group, 14 kHz and >20 kHz, respectively. Forward masking experiments indicated that their remarkable high-frequency hearing derives from the basilar papilla. Together with published anatomical and physiological data, these findings suggest a unique division of labor between groups of sensory cells within the hearing organ. Rather than having only strictly group-specific frequency ranges, amniote vertebrate hearing is clearly influenced by species-specific physical and ecological constraints.

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P 36

Sensory cells without innervation in the auditory papilla of geckos

Most lizards have good hearing when tested electrophysiologically but auditory guided behaviours are only known anecdotally. Geckos are a notable exception because they vocalise, calls of some species of pygopod geckos having exceptionally high frequencies, up to about 20 kHz. Geckos also have the most specialised basilar papillar (cochlear) structure among lizards. They show a separation into morphologically distinct low- and high-frequency segments (below and above approx. 1 kHz) which is typical for all lizards. In addition, in geckos, the high-frequency segment is further subdivided into two areas, running parallel along most of the papilla's length and separated by an axial hiatus. Our model had suggested a subdivision of the above-1kHz hearing range between pre- and postaxial areas, but there are conflicting data on whether preaxial hair cells are actually innervated or not. We have examined the innervation of the basilar papilla in pygopod geckos, using immunohistochemistry. Anti-NF200, a generic marker of neural processes, labelled fibres to the low-frequency papillar segment and the postaxial region of the high-frequency segment. Remarkably, the preaxial region was by-passed and appeared to receive no innervation at all. However, preaxial hair cells strongly labelled with anti-CtBP2, a marker of afferent synaptic ribbons. Anti-ChAT and anti-SV2, two markers of efferent terminals, produced label exclusively in the low-frequency papillar segment. Our data are consistent with a recent report on the Tokay gecko and confirm a remarkable regression of innervation in the gecko basilar papilla. While the low-frequency segment retains both robust afferent and efferent innervation, the postaxial hair cells of the high-frequency segment receive only afferent fibres and the preaxial hair cells are devoid of any innervation. This complete loss of innervation appears to be a recent evolutionary event, as the hair cells continue to form synaptic ribbons. It has been suggested that the preaxial hair-cell population is specialised for stimulus amplification. However, this would represent a unique case of a ”cochlear amplifier“ which is not controlled by efferent feedback.

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Bioacoustical techniques for invasive fish species management

Once an invasive fish becomes established, it is difficult to remove or manage the population without concurrent detriment to native species. We have been using bioacoustics to develop species specific methods to trap or halt the migration of invasive fish. A bioacoustic buoy has been developed to condition carp to associate low frequency (500 Hz) pure tone stimulus with food reward. The active (sound) and control buoys (silent) are placed two to ten meters apart. Carp quickly learn to locate the sound source, and correctly choose the active buoy over 90% of the time within one week of training. Following two weeks of twice daily conditioning, carp were tested at one, two and five month intervals to determine the length to time they retain the conditioning. At all intervals, carp would consistently choose the active buoy without further reinforcement. The eventual goal is to condition carp in the field and move the buoy to shallow waters to allow for netting and extirpation. We are also developing a hydrodynamic barrier using a combination of bubble curtains and current to impede adult carp migration into shallow spawning areas. Additionally, a bioacoustic fish trap that will mimic the sounds produced by male round gobies is being tested to capture female fish in the Duluth-Superior Harbor. One of the main reasons the round goby is a successful invader to the Laurentian Great Lakes is that is spawns throughout the spring and summer compared to yearly spawning for native fish. The removal of gravid females throughout the year, should reduce round goby populations.

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533 **P 38**

Hearing Capacity and Development of the Auditory Function in Zebrafish

Zebrafish (Danio rerio) has become an important vertebrate model for hearing research because of a combination of powerful genetics, excellent embryology, and exceptional in vivo visualization in one organism. However, it is still not clear how well they can hear and how their auditory function develops. In this study we obtained an audiogram of adult zebrafish using the acoustic evoked potential method. We also developed a classical conditioning method to assess the auditory ability of larval zebrafish from three days to sixty days post fertilization. Larval zebrafish movement was recorded by a high-speed camera and analyzed using MaxTRAQ 2D and MaxMATE software. Auditory thresholds were obtained at 200 and 500 Hz for the zebrafish larvae of different ages. We found that adult zebrafish have similar auditory thresholds to the goldfish (Carassius auratus), which is consistent with hearing ability of otophysans that possess auditory accessories for the enhancement of sound pressure detection. Hearing sensitivity of the zebrafish increases with age from hatching to two months old with a fast improvement of hearing sensitivity during the first two weeks post fertilization. The relationship between the auditory functional development and auditory structural growth will be discussed. Supported by NIH/NIDCD R21DC003275 and University of Miami College of Arts and Sciences Gabelli Fellowship to ZL.

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P 39

A non-taut tympanal membrane sustains narrow band resonance in the New Zealand tree weta ear (Anostostomatidae, Hemideina)

The New Zealand tree weta (Hemideina thoracica, Ensifera: Anostostomatidae) has tympanal ears located on each of the prothoracic tibia. Unconventionally, the tympana are non-taut, presenting membranous processes bulging out from the tibial cuticle and many loosely suspended ripples. Another unusual character is the sclerotized and pigmented oval region, which is found only in some ensiferan species of Tettigoniidae and in Haglidae. This study used microscanning laser Doppler vibrometry to determine how such a tympanal membrane vibrates in response to sound, and whether the sclerotized region plays a role in hearing. The tympanum displays a single resonance set at the calling frequency of the male tree weta. This unusual example in insects tympanum acting as a filter to impose narrow band frequency tuning on the ear. Both tympana resonate in phase with the stimulus and with each other. Histological sections show that the tympanum is divided into two distinct regions. The sclerotized oval lies in the middle of a thickened region and is surrounded by a transparent and uniformly thin region. The thick region constitutes the thickest tympanal cuticle reported for any insect ear, and appears to act as a damping mass on the oscillation of the thin region. It is hinged dorsally to the tympanal rim and thus resembles the model of a "hinged flap". We present a new model showing how the thick region confers a mechanical gain onto the activation of the crista acustica sensory neurons during the sound-induced oscillations.

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517 **P 40**

The neural basis of active hearing in the mosquito Aedes aegypti

Male mosquitoes are faced with the difficult task of finding females in order to mate on the wing, and do so by detecting the low-intensity, low-frequency sounds produced by her wing beat. Hearing in mosquitoes is mediated through their antenna that respond to nanoscale mechanical displacements, which in turn initiates an electrical response in Johnston's organ (JO), the auditory sensory organ housed at the base of the antenna. Johnston's organ is arguably one of the most complex hearing organs found in insects, with almost 16 000 sensory cells in male mosquitoes, and 7500 in females. These sensory cells, organized into scolopidial units arranged radially throughout JO, have a dual sensory and motor function to both detect and contribute to the mechanical vibration of the antenna. This active sensing is mediated by synchronization between sensory cells, which reveals itself through the twice-frequency forcing of sets of neurons. We recorded this twice-frequency forcing extracellularly in local subsets of scolopidia in the mosquito species Aedes aegypti. Using a Hilbert transform to calculate the instantaneous frequency and phase of the neural response in the time domain, it was revealed that neurons located in the distal region of JO exhibited greater firing at twice the sound stimulus frequency than those located proximally. As the sound stimulus intensity was increased, so too did the proportion of cells firing at twice the frequency, suggesting entrainment to generate coherent oscillations. This is in accordance with a recent model of the active hearing process in the mosquito JO that found that scolopidia located distally provide the largest contribution to the mechanical amplification of the antenna through the entrainment of the neural response to twice the stimulus frequency. Our findings provide further evidence that this active amplification is a peripheral phenomenon mediated by the neurons in JO.

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P 41

Arrays of sensing and actuating neurones for active invertebrate hearing

Hearing is the mechano-electrical transduction of sound energy. In general, sound waves force some mechanical sensor to oscillate, the movement of which in turn is sensed by mechanosensory neurones. Mammals and some invertebrates use metabolically active processes to improve their hearing capability. In these cases, hearing is generally a nonlinear process relying on the exquisite sensitivity of sensory neurones and force generation from molecular motors. Arrays of these sensor/actuator units can theoretically be used to provide nonlinear signal processing useful for hearing. We describe a generic model for an ensemble of active sensors coupled to an external passive sensor, and show how a specific mathematical relationship, namely a 2:1 resonance, between the stimulus frequency and the neuronal refractory period can yield marked nonlinear features such as amplitude-dependent gain and hysteretic amplification. The results of this study are in excellent agreement with experimental observations into a model active hearing system – the mechanical response of the male mosquito antenna to the female wingbeat sound.

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Auditory tuning in Drosophila melanogaster: dependence on amplitude and female mating status

Drosophila melanogaster has long been known to use acoustic communication in courtship. In a sequence of behaviours spanning the range of sensory modalities, a male sings to a female by extending and vibrating one wing in attempt to induce her to mate. Not all males are successful and what constitutes a 'good' song remains unclear. This may in part be due to the complex non-linear dynamics of the D. melanogaster auditory system. Drosophila use Johnston's organ, located in the second segment of the antenna, to transduce mechanical displacement of the arista/funiculus segment into neural impulses. This system does not respond passively to acoustic inputs. Instead, the resonance of the antenna is actively changing, depending on the intensity of acoustic forcing, effectively altering the stiffness of the arista/funiculus. This response is generated by the mechanical action of the mechanosensory neurons themselves and constitutes a type of nonlinearity unusual amongst auditory systems. In the context of acoustic communication the function of this nonlinearity is not yet understood. Importantly, song amplitude is a factor that has been difficult to determine in Drosophila courtship due to methodological constraints. Using a broader dynamic range, the nonlinear mechanical response is characterised using several methods of acoustic actuation (loudspeaker, point source). Results indicate a sigmoid relationship between input sound amplitude and resonant frequency of the antenna and an increase in gain at lower amplitude inputs. Moreover, it appears that there is a small but significant change in the stiffness of the antenna attributable to mating. In effect, it takes more energy for mated females to elicit the same mechanical response as virgin females. It is known that sex peptide contained in the male ejaculate modifies behaviour in mated females. Further experiments are required to establish whether the effect of sex peptide extends to the auditory neurons involved in generating active mechanics in female hearing.

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369 **P 43**

Active process tunes ears to songs in Drosophilid flies

For almost a century, the mating behavior of Drosophilid flies has served as a model system for studying animal communication. During courtship, a male fly walks next to a female and vibrates one or both of his wings to produce a complex, multi-frequency sound signal. These courtship songs mediate mate and sex recognition and– if successful–increase the likelihood of copulation. Successful songs have been shown to differ in their spectral and temporal composition across Drosophila species and the respective differences are deemed to reflect, and contribute to, reproductive isolation and speciation in Drosophilid flies. What evolutionary role, however, has fallen to the receivers of this acoustic communication system, i.e. the antennal ears of the females, has not been explored, yet. Here we report on a comparative study of sound emissions and receiver mechanics in seven Drosophilids of the melanogaster species group. We show that (i) the flies' antennal sound receivers display species-specific differences in their best frequencies, that (ii) the receivers' best frequencies correlate with high frequency components occurring in the conspecific songs and that (iii) sound receiver tuning is actively brought about by the flies' mechano-transducer machineries.

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Is there evidence of DPOAEs in the mechanical motion of the locust tympanum?

The locust tympanal organ is a sophisticated example of insect hearing. The tympanal membrane has previously been shown to analyse the frequency of incident sound, such that sound energy is funnelled into waves of motion across the membrane, travelling to frequency specific neurone groups. In vertebrates have been shown to indicate active hearing processes. The emissions, known as, are found in active auditory systems when the organ is stimulated with two sounds of different frequencies, f1 and f2. The origin of distortion-product Otoacoustic emissions (DPOAEs) in the mammalian ear is the hair cells of the cochlea. In some insects, including locusts, previous studies have found evidence of distortion-products recorded acoustically, but the experimental approach raises questions as to whether the results are actually driven by active hearing processes. The site of the DPOAEs is still not understood in insect ears, as insect hearing organs do not possess hair cells and were previously thought to have linear hearing. The experiments have been revised, altering the experimental set-up to an open system, with appropriate sound pressure levels. A laser Doppler vibrometer records the deflection of the locust tympanal membrane at specific points where the mechanoreceptor neurones attach. This allows the membrane to be measured directly for the presence of distortion-products, at the position of the neurones which are believed to be actively generating membrane sound emissions. Two species of locust are investigated, Schistocerca gregaria and Locusta migratoria. The measurement, or lack thereof, of distortion-products in the motion of the tympanal membrane is discussed and compared with previous studies.

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Picometre scale mechanics in the Cicada hearing system

As in many other cicada species, Cicadatra atra gather and form chorus centres. Within these aggregations, females have to select and localize a single male for mating. This task might be complicated by the presence of other species calling in the same area. Cicadas have tympanal ears, with a combination of the ear's tympanal membrane and its tympanal ridge component, acting to transducer sound into mechanical motion with frequency filtering. Inside the tympanal ear system the tympanal ridge is connected mechanically to a large number of mechanosensory neurons through a cuticular extension known as the tympanal apodeme. The in vivo motion of the tympanal apodeme of female Cicadatra atra has been measured using microscanning laser Doppler vibrometry in response to the motion of the tympanal membrane when driven by sound. These vibrometry measurements clearly show that the tympanal membrane's nanometre motion is over a magnitude greater than that of the tympanal apodeme at the point of mechanosensory neuron attachment. Also, the tympanal apodeme functions as a further mechanical frequency filter, improving the tympanal ridge's frequency filtering. Thus the frequency band of vibration at the mechanoreceptor neurons is solely that of the male calling song. This study therefore enhances our fundamental understanding of the mechanical links between the external ear of the cicada and its sensory cells.

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391 **P 46**

Parallel processing in the auditory center of the honeybee brain

Honeybees detect airborne vibration by means of Johnston□fs organ (JO), located in the pedicel of each antenna. The sensory neurons of the JO send their axons to three distinct areas of the bee brain, the dorsal lobe, the dorsal subesophageal ganglion (DL-dSEG), and the posterior protocerebral lobe (PPL). The morphological and physiological characteristics of interneurons arborizing in these areas have been studied by intracellular recording and staining. The interneurons having arborizations in the DL-dSEG (DL-dSEG-Ints) and those in the PPL (PPL-Ints) have been identified. The DL-dSEG-Ints are categorized into two types, the DL local interneurons and the DL output neurons projecting into the other neuropile region. The dorsal lobe-interneuron 1 (DL-Int-1) is one of the DL local interneurons densely arborized in the DL-dSEG and the dorsal lobe- interneuron 2 (DL-Int-2) is one of the DL output neurons projecting into the lateral protocerebrum. DL-Int-1 responds to vibratory stimulation applied to the JO in either on-off-phasic excitatory or tonic inhibitory patterns, while DL-Int-2 responds to the same stimulation in tonic excitation. One of the PPL-Ints, posterior protocerebral lobe- descending neuron 1 (PPL-D-1), has dense arborizations in the PPL and sends their axons into the VNC. The PPL-D-1 responds to vibratory stimulation and olfactory stimulation simultaneously applied to the antennae in a long-lasting excitatory pattern. These morphological and physiological results suggest that there are at least two parallel pathways for vibration processing through the DL-dSEG and the PPL. The possible roles of the parallel pathways on the vibratory information processing in the primary auditory centers will be discussed.□@Supported by Grant-in-Aid for Scientific Research (C), Grant Number: 22570079.

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636 **P 47**

The Role of Spatial Release from Masking for Sound Source Segregation in the Acoustic Parasitoid Fly Ormia ochracea

A fundamental task in hearing is to recognize and locate behaviourally salient signals to their correct source despite complex acoustic conditions that may mask relevant information. Many acoustically communicating animals often show improved source recognition and localization when a signal of interest is spatially separated from a source of background noise, thus demonstrating a phenomenon known as spatial release from masking. Females of the acoustic parasitoid fly Ormia ochracea depend on directional hearing to locate singing crickets (Gryllus rubens) that they use as hosts for the development of their young. Male crickets are often distributed in dense aggregations, and such conditions can pose a challenge for Ormia in locating individual hosts. We investigated the ability of Ormia to localize cricket signals that were broadcast against background noise in two phonotaxis experiments. In one study conducted within the natural habitat of Ormia in Florida, flies were presented with two simultaneous host cricket signals from speakers that varied in the degree of angular separation (20° and 180°) relative to the fly's forward heading. For both angles most females selectively localized one speaker, thus demonstrating an ability to maintain a correct search path once a specific sound source has been recognized. In a second study conducted in the lab, female flies were presented simultaneous choice of two sounds (cricket call vs. broadband noise) under more extreme angular separations (6° and 180°). In addition, the cricket signal was broadcast at different sound intensities, thus altering the signal-to-noise ratio (SNR). The accuracy of orientation by females was not affected by angular separation; however, flies responded with shorter latencies for both larger SNR's and the 180° angular separation treatment. These results demonstrate that Ormia females show little effect of masking at the SNRs tested, and successfully localize individual cricket hosts within the complex spatial environment that characterizes G. rubens populations in Florida.

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Evidence for dynamic dendritic compartmentalization of adapting mechanisms in an auditory neuron.

TN-1 is an auditory neuron that detects changes in auditory scenes in tettigoniids. TN-1 adapts rapidly to fast pulse rates, firing only a burst of spikes at the beginning of the stimulation. The adapted state persists while the sound stimulation lasts. However, TN-1 responds to transient sound pulses presented during fast pulse rate stimulation if the carrier frequencies are sufficiently different. Pharmacological studies revealed two post-synaptic adaptation mechanisms: a transient calcium-mediated mechanism and a slow, tonic sodium-mediated mechanism. Due to the tonotopic organization of the auditory neuropile, different stimulus frequencies stimulate different dendritic regions of TN-1. Using intracellular calcium and sodium imaging, we test the hypothesis that both post-synaptic adaptation mechanisms are limited to the dendritic regions stimulated by the afferents activated by the stimulus frequencies, while un-stimulated regions of TN-1 remain sensitive. We confirmed that: 1) fast pulse rate stimulation evoked transient calcium concentration increases in both dendritic and axonal regions; stimulation with slow pulse rates led to a gradual accumulation of calcium in both regions, 2) fast pulse rate stimulation tonically increased the sodium concentration within TN-1 dendritic regions only; slow pulse rates did not result in a significant increase of the sodium concentration, and 3) low frequency stimulation increased both the calcium and sodium concentrations in rostral dentritic regions, while high frequency stimulation resulted in increases of both ions in caudal dendritic regions. This last finding agrees with the tonotopic organization of the auditory neuropile. We conclude: 1) that main mechanisms underlying TN-1 adaptation remains compartmentalized in the stimulated part of the dendrite, and 2) that the dendritic compartments are dynamically formed, depending on the carrier frequency of the stimulus.

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P 49

Slow adaptation currents contribute to spike-response variability in a sensory neuron

Channel noise is a dominant intrinsic noise source in sensory neurons and can account for variability in the timing of action potentials. To directly measure the noise characteristics of the underlying ionic currents, somatic recordings are required. These are often hard to achieve without damaging the sensory transduction machinery. In this study, we therefore introduce an indirect approach to assess the stochastic dynamics of sensory neurons based on interspike interval statistics of the spike train responses. Spike responses of receptor cells were recorded intracellularly from auditory nerve fibres of Locusta migratoria during simultaneous acoustic stimulation with pure tones of various intensities. The obtained interspike intervals (ISIs) show high variability with CVs up to 0.8 depending on sound intensity. With increasing spike frequency the shape of the ISI histograms changes from an inverse Gaussian to a peaked probability density. Additionally, the ISI correlations show a shift from slightly negative values to positive coefficients with increasing spike rate. By means of simulations of single-compartment conductance-based models we tested different assumptions of possible noise sources which could account for the observed transitions of the ISI histogram shape and correlation. Simulations of individual channel noise sources revealed inverse Gaussian ISI distributions for fast ion channel fluctuations, like from the mechanosensory receptor channels, while simulations of the slow changing ion channels mediating spike-frequency adaptation resulted in peaked probability densities. Mixed case studies where both fast fluctuations and adaptation channel noise are present showed a smooth transition between the two limit cases which is in aggreement with our findings from the locust receptor cell responses. This indicates that higher-order statistics can be used to distinguish different kinds of noise sources.

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384 **P 50**

EFFECTS OF ACOUSTIC TRAUMA ON AUDITORY FUNCTION IN DROSOPHILA MELANOGASTER

The Johnston's organ in the Drosophila antenna has been intensively studied as a genetic model for auditory function. Here we examine the immediate and longer term effects of acoustic trauma on the electrophysiology and morphology of Johnston's organ. We show that acoustic stress results in reduced auditory function that correlates with smaller mitochondria in Johnston's organ neurons.

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Cool Tools !

375 **P 51**

A new method to obtain 3D information on dye filled neurons in the CNS: spinal neurons in young Xenopus laevis tadpoles

Most studies of neuron morphology use reconstruction from image stacks after filling with fluorescent dyes. However, very small neuronal axons and dendrites filled with non-fluorescent markers like neurobiotin can be difficult to photograph. To investigate the anatomical development of functional networks in the hatchling Xenopus laevis tadpole we have devised a new method to measure 3D information on neuron morphology and position whole neurons and their axonal projections in 3D space within the nervous system. Using spinal neurons filled with neurobiotin during electrophysiological experiments the following questions were asked: what is the morphology of each individual neuron? What is the neuron's position in 3D space within the nervous system? Can we define possible contact points between axons and dendrites? Neurons were viewed in whole mounts of the tadpole CNS under a Nikon Optiphot microscope, using a DeltaPix camera. Specimen position was controlled by a Scientifica PatchStar micromanipulator and LinLab software which allowed the 3D co-ordinates of neurons and the CNS to be recorded 3D co-ordinates were recorded at successive diameter points of neuronal somata, dendrites and axons, perpendicular to the long axis of the structure being measured. Measurements taken were converted into .swc format, used by various commercial and freeware neuron morphology programs such as Neurolucida and NeuRomantic. The .swc format provides 3D co-ordinates of each diameter midpoint, radius, directional connectivity of each point and an identification code identifying the type of structure being measured. By generating numerical descriptions of neuron shapes and their positions within 3D space, we can normalise all specimens to the same origin point and thus overlay individual specimens in order to build a virtual CNS. This method provides an economical new approach to defining 3D morphology of non-fluorescent dye filled neurons.

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Novel Electromyogram (EMG) based mechanism for flight control

Extensive studies about neural mechanisms involved in insect flight control have been carried out. Integration of salient sensory cues with ongoing motor activity plays a significant role in adaptive control of locomotion. During flight, inputs received by an organism through sensory organs are processed by the central nervous system (CNS) and the integrated output thus obtained plays a significant role in controlling the wing phase shifts and flight muscle depressor asymmetries associated with adaptive flight maneuvers. The resulting maneuvers, in turn, bring a change in the insect's sensory environment, thereby closing the feedback loop. Research on insect flight has been carried out using immobile preparations (tethered) and mobile preparations (free flight – untethered). Insects such as locusts and moths exhibit pertinent wing phase shifts and asymmetries in depressor muscles. A feedback circuit that utilizes asymmetrical timing of bilateral depressor muscles, the forewing first basalars (m97), of the locust to close a visual feedback loop in a computer-generated flight simulator is presented here. The circuit converts the Electromyographic (EMG) time difference between left and right m97s to analog voltage values. Analog voltage values can be acquired using an open-loop experimental protocol (visual motion controlled by the experimenter), or can be used to control closed-loop experiments (muscle activity controls the visual stimuli) experiments. Tests with locusts, indicate that it was possible to detect the spike time difference and convert it to voltage values, which controlled the presentation of a stimulus in a closed loop environment. The feedback circuit presented here may be used in conjunction with the flight simulator(s) to understand the neural mechanisms involved in control of insect flight and provide further understanding of general mechanisms of neural control of behaviour.

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626 **P 53**

Imaging the fly brain using micro CT

Virtual 3D models are powerful tools for studying the functional organization of complex anatomical structures. Traditionally, the underlying data were acquired from fixed and sectioned material, involving excessive preparation times and inevitable tissue distortions. A candidate technique to overcome these problems is X-ray micro tomography (micro-CT). Recent technological advances, including improvements in contrast enhancing stains as well as image processing, now allow for its successful application to soft tissue preparations. Here we present a 3D virtual reconstruction of the fly brain and head capsule. We fixed a male blowfly (Calliphora vicina) in ethanol and elemental iodine and scanned the specimen using a micro-CT scanner at the Natural History Museum, London. Cuticular structures and soft tissues, such as muscles, nerves and the brain were clearly visible and distinguishable due to differences in radio-opacity. The specimen was reconstructed using 3D rendering software to generate an immediate visualisation. From the 3D reconstruction we were able to manually segment major brain regions, such as optic lobes, mushroom bodies and the central complex as well as the head capsule. Significant advantages of micro-CT over traditional sectioning methods include mitigation of artefacts due to tissue distortions, avoiding data loss due to sectioning, and its rapid speed (approximately 60-120 minutes) as well as its relative simplicity and the non-toxicity of the fixation. The high throughput makes this technique well-suited for studies on natural variability, while the possibility to simultaneously distinguish between a wide range of different tissues and structures allows for a comprehensive 3D model of the animals' functional anatomy. The potential to visualise sensory organs, nervous tissue, muscles and skeletal structures is an ideal prerequisite for micro-CT to become a powerful technique in neuroethology research.

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P 54

Anatomic analysis of Gal4 expression patterns of the Rubin line collection: the central complex

We present an overview of the Rubin GAL4 driver line collection (Pfeiffer et al. PNAS 2008). Approximately 6,000 lines have been imaged in the adult brain and VNC, out of an anticipated 7,500. After eliminating the 35% of lines with either very broad or no expression, we annotate the expression patterns of the remaining lines using a detailed, controlled vocabulary developed over the past two years by an international consortium (Ito et al, submitted). The annotation itself is accomplished in three parallel pathways: (A) assignment of arborization patterns to neuropil regions by a human annotator, (B) automatic (computer based) annotation and (C) high-resolution expert annotation for specific areas. The fusion of these approaches (into a searchable database) will provide a detailed view of the expression patterns of the lines in this collection and will facilitate the design of new experiments, including the use of intersectional and stochastic labeling mehtods. We describe a set of efficient software tools for the manual annotation of large image datasets, as well as the principles of automatic annotation and its first results. Finally, we illustrate the use of these tools by presenting an initial analysis of expression patterns in the central complex.

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Serial Block-Face Scanning Electron Microscopy: A Method for Delineating Neural Circuitry of a Behavior

We exploit the capabilities of the Serial Block-Face Scanning Electron Microscope (SBFSEM) to address the issue of the relationship of peripheral sex differences and the structure and organization of a simple circuit, the rapid copulatory circuit (RCC), of the male Western Mosquitofish Gambusia affinis (hereafter, Gambusia). Our research objective is to develop for the RCC a connectome, a summary of the structure of a neural network and its synaptic connections between and among spinal cord motor neurons. Despite advances in light microscopy, electron microscopy continues to be the only imaging modality capable of seeing every axon, dendrite, and synaptic connection within a volume of neuropil. The most promising alternative for wide-field microscopy imaging at the electron microscopic level is SBFSEM because: sections are not lost or distorted; optical sections within image stacks are perfectly aligned; and large wide-field volumes can be imaged without significant operator involvement. In addition, SBFSEM provides resolution that is sufficient to trace even the thinnest axons and to identify synapses, revealing in Gambusia spinal cord: extensive dendritic branches and arbors as well as dendritic spines and axons terminating on the soma of motor neurons. To elucidate the general rules by which complex local and global neural circuits operate, neuroscientists must be able to simultaneously examine large circuits in their entirety and trace fine dendrites, axons, and synaptic connections. SBFSEM meets that need. However, as with other cutting-edge technology, SBFSEM not only greatly enhances work in a field but also requires the development of additional tools, in this case, processing methods and image analysis and storage capabilities. Understanding the capabilities and limitations of new enabling tools such as SBFSEM is essential in addressing specific research questions as well as larger and fundamental questions. This research was support supported by NIH/NS30405.

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Photoactive Cyclases (PACs) as optogenetic tools for spatiotemporal manipulation of cAMP signaling.

During synaptic plasticity and memory storage, intracellular signaling processes are rapidly activated by the action of neurotransmitters on ionotrophic and metabotrophic receptors. These intracellular signaling pathways alter neuronal function by rapidly changing levels of intracellular calcium and cAMP that, in turn, activate a cascade of kinases involved in the regulation of gene expression and de novo protein synthesis. Whereas these biochemical processes occur very rapidly within specific populations of neurons, the genetic and pharmacological tools used to study them in vivo have poor spatial and temporal resolution. Genetic knockouts result in the loss a particular gene for the entire lifetime of an organism and regulated transgenic expression or conditional knockouts act over the time course of hours to days. In addition, pharmacological reagents often lack the cell specificity needed to selectively target signal transduction pathways in neurons. Here we show that Photoactive Cyclases (PACs) rapidly and transiently alter cellular cAMP levels in a spatially restricted manner thereby overcoming these limiting issues. To determine the experimental benefits and limitations of PACs we used a renal secetion assay and show that secretion is bidirectionally regulated by cAMP depending on the intracellular conentration of the second messenger. Moreover, we present alternative strategies to improve experimental use of PACs.

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537 **P 57**

The Flywalk â€“ a new paradigm to screen odor-guided behavior in Drosophila

Due to the astonishing functional similarity of the vertebrate olfactory system and that of Drosophila and due to the wide range of genetic tools established for this fly, Drosophila melanogaster has become a model system for olfaction. So far Drosophila's behavior was either investigated in mass experiments like trap assays and T-mazes or in time-consuming experiments using single flies. Here we present a new paradigm in which we individually track 15 freely walking flies in parallel small-sized wind tunnels (length, 17cm; diameter, 0.8 cm, wind speed, 30 cm/s). The wind tunnels are connected to a computer controlled stimulus device that can handle and mix up to 8 odors, and produces odor pulses, well defined concerning odor quality, quantity and stimulus duration. As the automatic tracking system is informed about the timing of the pulse, the wind speed and the position of each fly, it can calculate the point in time, when the pulse reaches the individual flies. By comparing the flies' movements directly before and after the stimulus we found odor-specific responses (up- or downwind runs) with again odor-specific response latencies ranging from 0.6 to 1.5 seconds. During a 3-hour lasting experimental session every individual fly encounters a total of up to 180 odor stimuli. Hence, this paradigm for the first time allows screening of large numbers of individual flies for their olfactory sensitivities, odor preferences, and response latencies. Furthermore the system allows screening of large sets of odors for their attractiveness to Drosophila. Using this system we characterized individual flies' responses to food- and sex-linked odors like balsamic vinegar (and its individual components) and CVA, as well as repellents like benzaldehyde.

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Genetically driven changes of synapse number in local interneurons modify olfactory perception in Drosophila.

It has been proposed that inhibitory synapse loss in cerebral cortex neurons could be a key factor in schizophrenia, whereas the loss of excitatory synapses might play a role in several types of dementia. However, a direct cause-effect between synaptic ratio and perception has not been demonstrated to date. In the olfactory glomeruli of insects, changes in the number of synapses are related to changes in perception. Experimental alterations in this number modify the attraction/repulsion behavior of Drosophila to odorants. We overexpressed components of the PI3K/AKT/GSK3 signaling pathway to induce a cell autonomous reduction of the number of synapses –without affecting cell number - in two defined subsets of local interneurons of the olfactory glomeruli, and measured the behavioral effects on olfactory perception in a choice assay. The reduction of one subset of synapses, mostly inhibitory, elicited repulsive responses to all odorants and concentrations tested, while the reduction of another subset, mostly excitatory, leads to a shift towards attraction. Functional data based on calcium imaging show the expected changes of neuronal activity in genetically modified antennal lobes when stimulated by odorants. Most interesting, the simultaneous reduction of both synapse subsets restores normal perception. Using genetic tools for space and temporal control of synapse number decrease, we show that the perception effects are specific to the local interneurons, and independent from putative major structural changes elicited during development. Also, when synapse reduction was generated on other neuronal types different from the olfactory pathway, the mushroom body neurons, the perception profiles remained as in controls. These findings demonstrate that synapse loss is at the origin of sensory perception changes, and that the functional ratio of excitatory/inhibitory local interneurons of the antennal lobe plays a key role in the normalcy of odorant perception.

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Courtship decision making is regulated by signal equilibrium between two olfactory pheromone pathways

How does an aminal extract salient information from its complex and variable environment? In fruit fly, Drosophila melanogaster, cis-vaccenyl acetate (cVA), a product of the male ejaculatory bulb, works as a courtship inhibitory pheromone. Recently we found that a male was capable to detect as low cVA on a target fly as one thousandth of its own and exhibited a reduced level of courtship. How does a male detect such a tiny amount of cVA without being distracted by his own cVA? In order to examine how pheromonal information is processed in the olfactory system, we performed physiological analysis using an activity-sensitive fluorescent marker, synaptopHluorin and found that the pheromone-sensitive ORNs received cholinergic excitatory input to their presynaptic terminals. Blocking expression of the acetylcholine receptor in either courtship stimulatory ORNs or inhibitory ORNs diminished cVA-responsive courtship suppression, suggesting that lateral feedback modification of ORNs is critical for pheromone coding. Intriguingly, however, co-suppression of the excitatory feedback in both types of ORNs rescued the cVA-response defect, indicating that the behavioral output is determined by the signal equilibrium between the two olfactory pheromone pathways. We also found that prolonged exposure to purified cVA caused an enhanced level of subsequent courtship activity, indicating that there is adjusting mechanism that maintains the basal balance between the two groups of ORNs at neutral level. Becasue of this background-noise canceling mechanism, a male might be able to ignore his own smell and lead to an appropriate courtship decision.

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Physiological and morphological characterization of four classes of local interneurons in the Drosophila antennal lobe

The Drosophila antennal lobe (AL), with the power of neurogenetic tools, has become an excellent model for studying olfactory processing mechanisms. Although the neural circuit of the Drosophila AL has been intensively studied at both the input and the output level, the internal circuit is not yet well understood. An unambiguous characterization of local interneurons (LNs), which are ideally positioned to increase computational capabilities of odor information processing in the AL, is essential to remedy this lack of knowledge. We used whole-cell patch-clamp recordings in situ combined with detailed morphological analysis to characterize LNs at the single neuron level. Four classes of LNs were characterized with class specific electrophysiological and morphological properties. Each class of LN displayed unique characteristics in intrinsic electrophysiological properties, showing differences in firing patterns, degree of spike adaptation and amplitude of spike afterhyperpolarization. Notably, one class of LNs had characteristic burst firing properties, while the others were tonically active. Morphologically, neurons from three classes innervated almost all glomeruli, while LNs from one class innervated a specific subpopulation of glomeruli. Three-dimensional reconstruction analyses revealed general characteristics of LN branching patterns and further differences in dendritic density and distribution within specific glomeruli between the different classes of LNs. The current study provides a systematic characterization of olfactory LNs in Drosophila and demonstrates that a variety of inhibitory LNs, characterized by class-specific electrophysiological and morphological properties, construct the neural circuit of the AL.

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P 61

Perception of odor mixture in Drosophila imago

Mixtures of odorants are perceived differently from their components. We are studying responses to binary mixture at the level of olfactory receptor neurons (ORNs) in sensilla basiconica. Responses to a mixture of two odorants are either simply additive or exhibit agonism and antagonism. Peripheral interactions at the level of ORNs significantly modify the antennal representation of the odorants. 2-3 Butanedione, for example, is a strong agonist of acetone. It increases the firing rate by about 151%. Butanedione- acetone thus evokes a sharp peak of firing instead of broadly tuned responses. Agonism shows a sharp peak and occurs at a fixed ratio of butanedione to acetone independently of absolute concentration. In the behavioral test with single fly, flies exhibit robust increase in response. The peak of agonism corresponds to peak of attraction. Or83b blind mutants do not exhibit agonism. Agonism provides a novel method of coding the responses of Drosophila to binary mixtures.

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P 62

Dual pathways to process general odors in the cockroach brain

The cockroach, Periplaneta americana, is equipped with excellent capabilities of olfactory discrimination and learning, and has been drawn attention as one of the ideal animals to study neural mechanisms of olfactory communication. However, in contrast to neural processing of specific odors, such as sex-pheromone, relatively little is known about that of more general odors. Olfactory receptor neurons (ORNs) in antennal sensilla project to about 205 glomeruli in the antennal lobe (AL), and then they synapse onto a moderate number of projection neurons (PNs) and local interneurons. Anterograde dye injections into the antennal nerves revealed sensory axons supplying the AL are bundled into ten sensory tracts (T1-T10) and innervate two glomerular groups. The antero-ventral group, innervated by T1-T3, contains small oval-shaped glomeruli, whereas the postero-dorsal group, innervated by T4-T10, contains large glomeruli with various shapes. In the cockroach antenna, three distinct types of olfactory sensilla were morphologically identified, and each sensillum housed a few ORNs. Axons of ORNs in basiconic sensilla project to the antero-ventral group glomeruli and those in trichoid and grooved basiconic sensilla project to the postero-dorsal group glomeruli. This result suggests that olfactory signals processed in distinct sensilla types are mapped in distinct glomerular clusters. Next, we focused on the AL output pathways. In the cockroach brain, two morphological types of uniglomerular PNs were reported: type 1 PNs (PN1s) and type 2 PNs (PN2s). PN1s and PN2s are arborizing in single glomeruli in the postero-dorsal and antero-ventral group, respectively. Intracellular staining revealed the axons of both types of uniglomerular PNs terminate in both the calyces and the lateral horn, although there are no overlaps between axon terminals of PN1s and PN2s. These results suggest that information about general odors is processed by two parallel pathways anatomically segregated.

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Temporal Integration for Gain Control in the Moth Olfactory System

Sensory input is processed in the brain, and elucidating the temporal dynamics of neural activity is crucial to understand neuronal information processing. In the insect olfactory system, the gain control mechanisms are important in shaping the odor responses of second order neurons, the antennal lobe projection neurons (PNs). However, precise temporal dynamics and behavioral relevance of the mechanism remain poorly understood. One reason why these questions have been difficult to resolve is the difficulty of delivering olfactory stimuli with millisecond precision. To address this, we genetically introduced channelrhodopsin-2 (ChR2), a light-gated ion channel isolated from green algae into the olfactory system of an insect model system for olfaction, the silkmoth. We generated a transgenic silkmoth line expressing ChR2, under the control of the promoter sequence of the gene encoding the olfactory receptor for bombykol, the major sex pheromone in the silkmoth. By using brief pulsed photostimulation, we demonstrated light-activated pheromone orientation behavior in the transgenic moths, and succeeded in controlling the number of spikes in olfactory receptor neurons (ORNs) expressing ChR2. Paired pulse photostimulation revealed that there is a defined time window in which temporal integration of ORN activity occurs in PN, and this temporal integration in PN seems to play an important role in gain modulation of olfactory circuit. A direct behavioral correlate of this gain modulation was found as responsiveness to pheromone increased odor stimulus conditions resulting in temporal integration in the PN. Our results indicate that the temporal integration in PN provides an important gain control function on a behaviorally relevant timescale.

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P 64

Central processing of a mixture of pheromone and plant odor in the MGC of Agrotis ipsilon

Encountering food sources and possible sex partners is a crucial part in an animal"s life. Moths navigate towards these two goals mainly by using their olfactory sense. The chances of finding a sex partner should increase the closer the animal gets to food sources, thus the processing of the two olfactory stimuli could be related. In this study, we analysed the effects of the sex pheromone and heptanal, a plant odour, and the mixture of the two in Agrotis ipsilon males. We investigated the responses to these odours and the anatomy of projection neurons (PNs) in the macroglomerular complex (MGC) in the primary olfactory centre, the antennal lobe (AL), by means of intracellular recordings and stainings. Our results revealed three possible responses to the sex pheromone: 1. a biphasic response consisting of an excitatory phase and an inhibitory phase 2. a tonic, excitatory response and 3. a tonic inhibitory response. Heptanal mostly elicited inhibitory responses, yet some biphasic and some tonic excitatory heptanal responses could be observed, some neurons did not respond to heptanal at all. The responses to the mixture mostly resembled a pheromone response suppressed by heptanal. In a few cases a hypoadditive odour interaction was observed, meaning that the responses to the mixture were very similar to the pheromone response. Synergism, i.e. the mixture response was stronger than the strongest response to a single component, could also be observed in a few cases. The main effect of heptanal on pheromone-responding MGC PNs seems to be inhibitory. As most pheromone responses are biphasic, we suppose that the inhibition in response to heptanal adds up with the inhibition present in the pheromone response and thus serves a better separation of temporally separated excitatory phases and a better separation of excitatory phases from the spontaneous activity of the neuron. This inhibition could also contribute to the synchronisation of excitatory phases of different neurons.

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A precise population code for attractive pheromone mixtures

Odors that elicit and guide animal behavior typically consist of multiple volatile chemicals. Much has recently been learned about how individual odors and mixtures are encoded, especially in the primary olfactory center (olfactory bulb in mammals, antennal lobe in insects). However, little is yet known about whether animals utilize higher-order features of odor mixtures, e.g. the relative proportions of components, or how these features are encoded in the brain. We report that the natural, 2:1 ratio between the two major components of the pheromone of a moth (Manduca sexta) is most effective in eliciting mating behavior, and is encoded in the precision of synchronous spikes produced by a population of cells in the antennal lobe of the animal. Males were more likely to exhibit mating behaviors to synthetic pheromone mixtures mimicking the natural 2:1 ratio than mixtures with altered ratios. We quantified the information about the ratio of components in the mixture available in various features of the output neurons of the Macroglomerular Complex (MGC), an assemblage of antennal lobe glomeruli responsive to the pheromone. Reliable information was available in the precision of synchronous spikes between pairs of PNs in the MGC. Additionally, pairs of neurons were found to belong to one of two populations: one population responded with more precise synchrony to mixtures with greater proportion of one component, the other to mixtures with a greater proportion of the other component. In this way, synchrony is most precise in the whole population when the natural, 2:1 ratio is encountered. This mechanism likely contributes to the behavioral preference for the natural pheromone mixture.

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Gain control of the sex pheromonal signal by local interneurons in the antennal lobe

Female sex pheromone components are the most important odor information for the male silkmoth (Bombyx mori). Their signals from the olfactory receptor neurons (ORNs) are sent to a set of specialized glomeruli, called macro glomerular complex (MGC) in the antennal lobe (AL), the first olfactory center in the insect brain. In the MGC, they are transferred to the projection neurons (MGC-PNs). Besides the MGC-PNs, local interneurons (LNs) also innervate the MGC. Until now, even the concentration-response characteristics of AL neurons to the pheromone components are not well understood. First of all, to investigate the response characteristics of the MGC-PNs and the LNs in the AL, we developed a method to selectively record the activities of defined types of neurons using Ca(2+) imaging. We injected Ca(2+) indicator via a glass micropipette by application of an electric current into the regions that the MGC-PNs or the LNs densely innervate. Local electroporation enabled us to selectively load the indicator into each population of neurons. Using this method, we simultaneously recorded the responses of dendrites and somata of the MGC-PNs to bombykol, the major sex pheromone component, to reveal how the intensity information of the pheromonal signals is transformed in the AL. The responses at both sites increased with increasing stimulus concentration. However, at higher concentrations, responses at the somata clearly decreased while responses at the dendrites still increased. We also recorded the responses of LNs to bombykol. Calcium in the LNs phasically increased locally in the MGC although the LNs usually innervate wide areas of the AL. LNs responded to bombykol above a rather high threshold coinciding with response decrease in MGC-PNs. After pharmacologically blocking inhibition to the MGC-PNs, the responses at the somata increased even at high stimulus concentration as well as at the dendrites. These results imply that LNs inhibit pheromone responses of MGC-PNs downstream of the ORN-PN synapses acting as a selective gain controllers.

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Multi-unit recordings in the dual olfactory pathway of the honeybee

Odors are encoded by the activity of olfactory receptor neurons (ORNs) that relay odor information to the primary olfactory centers in the brain, the insect antennal lobe (AL) or the vertebrate olfactory bulb. In the honeybee, neuronal information is conveyed from the AL glomeruli to higher integration centers in the mushroom bodies (MBs) and the lateral horn (LH) via two separate uniglomerular projection-neuron (PN) output tracts, the medial and lateral antennoprotocerebral tracts (m- and l-APT). This dual uniglomerular PN olfactory pathway to the MBs is a unique character of Hymenoptera (Kirschner et al. 2006, J Comp Neurol 499:933; Zube et al. 2008, J Comp Neurol 506:425). It is assumed that the dual olfactory pathway serves the assessment of quality, intensity and temporal structure of an odor stimulus at the level of the MBs. The MBs are known to be involved in higher-order processing like learning and memory. To investigate the role of the dual APTs in coding temporal properties that are relevant for coincidence detection at the level of the intrinsic MB neurons, the Kenyon cells, we analyze electrophysiological properties of both APTs by simultaneous recordings using multiple wire electrodes (adapted from Okada et al. 2007, J Neurosci 27:11736) and a customized highly accurate multi-unit recording setup. First results from dual tract recordings revealed that PNs from both tracts respond to the same set of general odors and pheromones. Recordings from m-APT units showed a high specificity for odor identity and complex excitatory and inhibitory responses to odor stimulation. In contrast, recordings from l-APT units were less specific for odor quality. M-APT neurons showed positive as well as negative correlations to rising odor concentrations, whereas responses from l-APT units, in most cases, were concentration invariant over the intensity range tested. Our present results support parallel processing of different properties of an odor stimulus via the two APTs. Supported by DFG, SFB 554 A8

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P 68

Neuronal correlates of colony recognition cues in ants

Ants discriminate colony members (nestmates) from members of different colonies (non-nestmates) by their colony odor, which consists of colony-specific hydrocarbons on the cuticle (label). During the recognition process, the label is compared to a neuronal template (label-template matching) and a mismatch will lead to aggression. The neuronal basis of label-template matching is unknown. In search for the neuronal correlate of the label, we retrogradely stained output neurons of the primary olfactory center of the ant brain, the antennal lobe (AL), and presented label-treated dummies. We measured neuronal activity in the upper half of the AL in response to both nestmate and non-nestmate label using calcium imaging with a CCD camera. Our results show that workers can perceive the label of their own colony. A template implemented simply as a sensory filter in the antenna, where olfactory receptor neurons are adapted to the ever-present nestmate label and only non-nestmate label is detected, is hence unlikely to be the main mechanism of nestmate recognition. Alternatively, sensory information might be specifically modified along the olfactory pathway to allow discrimination, with specific modifications that result in a template. We compare neuronal activity patterns elicited by nestmate and different non-nestmate labels using multi-variant statistics in search for specific modifications of neuronal representations. In order to map the neuronal activity in the whole AL, we use calcium imaging with a two-photon microscope and analyze the responses of glomeruli located in the lower part of the AL. Differences in the neuronal responses to the label in different parts of the AL might indicate that different mechanisms are realized in parallel to achieve efficient and reliable nestmate recognition. Funding: DFG SSB554/A6 & GSLS Würzburg

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427 **P 69**

Behavioural and sensory ecology of learning in butterfly-ant associations

The majority of lycaenid butterfly associates with ants during their larval stages. These associations are mainly mutualistic interaction, but range from non-ant associated to parasitism. In any associations, lycaenid caterpillars will have evolved a range of chemical signals to communicate with their ant partners. However, little is known about the sensory bases and evolution of chemical signalling in the lycaenid-ant associations. Using mutualistic (Narathura japonica) and non-ant associated (Lycaena phlaeas) butterfly species, we addressed how chemical signals from caterpillars are processed by the ants, and whether signals to ants are varied according to type of associations. In mutualistic association, we found that ants can learn to associate the nectar secretion with the cuticular chemicals of mutualist caterpillars, and increased attending behavior toward them. Such association via ant learning was not observed in non-ant associated L. phlaeas caterpillars. They have no nectar-secreting organ on their surface. In addition, learning experiments using artificial nectar and L. phaleas cuticular chemicals revealed that ants could not recognize their cuticular chemicals. Chemical analysis showed that cuticular chemicals of mutualist caterpillars were composed by complex mixture of various kinds of own (non-mimetic) hydrocarbons. On the other hand, non-ant associated caterpillars have simple set of hydrocarbons, mainly occupied by linear alkanes. From these results, we will discuss the evolution of chemical communication in the diverse lycaenid-ant associations.

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Sensory structure roles in odour-gated rheotaxis during navigation by the nudibranch mollusc Tritonia diomedea

Many animals use odour-gated rheotaxis to navigate, following the flow upstream to find odour sources. The nudibranch Tritonia diomedea uses odour-gated rheotaxis, however unlike faster moving animals it does so without any movement across the flow. Rather, the slugs turn and crawly directly upstream in attractive odour plumes, and directly downstream in aversive odour plumes. This suggests the slugs navigate by measuring flow direction and then choosing either positive or negative rheotaxis, dependent on odour type. Our goal is to test the roles of different sensory structures in the initial navigational turns inside odour plumes. Previous studies showed that the paired rhinophores detect odours and that upstream turns in the absence of odours depend on flow detection by the oral veil. We hypothesize that upstream and downstream turns in response to attractive and aversive odours could be generated in three possible ways: 1) reflex turns contingent on odour type and which of the rhinophores first detects the odour; 2) either positive or negative rheotaxis contingent on odour type and flow direction, both detected by the rhinophores; or 3) either positive or negative rheotaxis, contingent on odour type detected by the rhinophores and flow direction detected by the oral veil. To distinguish amongst these hypotheses, we stimulated rhinophores with prey, predator or control odour, either in the same direction or in opposition to ambient flow, as well as in still water. Preliminary results suggest that turns stimulated by predator odour are reflex responses. Slugs turn contralaterally away from whichever rhinophore detects the odour, regardless of flow conditions. Responses to prey odour were more variable, but are not reflexive ipsilateral turns. The relative roles of the oral veil and rhinophores in these turn responses are still unclear. These experiments are an important step in guiding further electrophysiological experimentation on the neural circuits underlying navigation behaviour in Tritonia.

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A neuroethological and molecular approach for characterizing the role of olfaction in the alarm reaction in piauçu fish (Leporinus macrocephalus)

Anti-predator behavior in Ostariophysan fishes is mediated by an alarm pheromone system that is thought to “warn” conspecifcs about predator activity. The current study was undertaken to determine the role of olfaction in the alarm reaction of piauçu performing lesions in olfactory bulb (ob) and quantifying the changes of c-fos expression. Neuroethological study consist in submit animals (sham, n=17; partial lesion of ob, n=14; total lesion of ob, n=12) to the open field test and measure the locomotion and behavior 10min after and before introduction of 1ml of conspecific alarm substance (CAS) or vehicle (VEH). For c-fos activity study, animals were sacrificed 90min before receiving 1ml of CAS (n=4) or VEH (n=4) and the ob were dissected to perform the Western blotting. VEH-treated animals did not alter ongoing behavior or significantly change locomotor activity. Sham and partial lesioned animals displayed freezing behavior and significantly decrease locomotion when exposed to CAS (p=0.014; p=0.007, respectively). In contrast, total lesion of ob did not alter ongoing behavior or significantly change locomotor activity. In addition, a significantly increase in c-fos expression was observed in animals treated with CAS relative to VEH (p=0.029). This finding is in accordance to the previous data who demonstrated that olfactory-impaired of fathead minnows did not recognize the CAS. However, this is the first time that a work demonstrated: (i) the total integrity of ob is not necessary to recognize the CAS and; (ii) the increased expression of c-fos in ob caused by CAS.

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P 72

Physiological state and odorant identity modulate modulation in the olfactory epithelium

In axolotls (Ambystoma mexicanum), a type of aquatic salamander, the terminal nerve contains gonadotropin releasing hormone (GnRH) and neuropeptide Y (NPY). Previously, we found that both peptides alter odorant responses in the olfactory epithelium: the effects of GnRH varied across the breeding season, while those of NPY were only observed in food-deprived animals. These findings suggest that responses to odorants are modulated in a context-dependent manner. We are now examining the effects of both peptides on responses evoked by various odorants in animals of both sexes in different reproductive and nutritional states. Specifically, we are using electro-olfactogram (EOG) recordings to measure responses elicited by food odorants as well as those from male and female axolotls. As a control we are using isoamyl acetate, an odorant with no biological significance to axolotls. To manipulate reproductive and nutritional state, animals are housed in separate rooms with different light and feeding schedules. For each EOG recording we measure baseline odorant responses; then bathe the epithelium with 1 μM NPY, 10 μM GnRH, or Ringer's solution (control) for 20 min; then wash off the peptide and record responses for another 40 min. Preliminary data from 29 animals suggest that the effects of both peptides vary with the animal's sex, reproductive and nutritional state, and the behavioral significance of the odorant stimulus. For example, in food-deprived axolotls GnRH suppresses responses evoked by both food and conspecific odorants. Conversely, NPY enhances responses to food odorants in food-deprived axolotls. In well-fed reproductive axolotls, the response to conspecific odorants is enhanced during the wash period after application of GnRH. However, in non-reproductive animals responses to all odorants are suppressed throughout the recording period when either GnRH or NPY is applied. These studies contribute to understanding how the vertebrate brain regulates sensory activity to emphasize stimuli that are most relevant to the animal's physiological state and behavior.

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Categorization of chemosignals within rodent medial amygdala: Patterned responses dependent on GABA and hormone action and on vomeronasal input

Chemical-communication signals from conspecifics and those used by heterospecific species activate immediate early gene responses in medial amygdala of male hamsters and mice. Signals that elicit behavioral responses from the receiver and can reasonably be considered biologically relevant for that species activate both anterior (MeA) and posterior medial amygdala (MeP) in both species. Non-relevant signals or electrical stimulation fail to activate posterior medial amygdala, with evidence for GABA inhibition from adjacent intercalated nucleus (ICN) (Meredith and Westberry 2004). Relevant stimuli differentially activate subregions of MeP (Samuelsen and Meredith 2009a) and subpopulations within those regions, including GABA-ir, GABA-Receptor-ir and Calcium-Binding Protein-ir cells. Despite anatomical evidence for main olfactory input to medial amygdala, chemosignal response there is unaffected by main olfactory epithelial ablation but is eliminated by vomeronasal organ removal (Samuelsen and Meredith 2009b). Some response to main olfactory input can be restored by intracerebral GnRH (Blake and Meredith 2010), or by sexual experience (Westberry and Meredith 2003). Androgen receptor (AR) expression in MeP is upregulated indirectly by chemosensory stimulation, leading to a 2-stage response, but medial amygdala response is lost after intracerebro-ventricular injection of oxytocin antagonist. These results suggest a complex synergy of sensory and hormonal inputs governing normal neural and behavioral responses to chemical communication signals. Supported by NIDCD grants DC005813, T32-00044.

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Unilateral integration of multimodal chemosensory information

Insects have developed unique chemosensory system to adapt themselves to various environments. In many species, chemosensory systems are necessary for feeding behavior. Both gustatory and olfactory systems are required to elicit appropriate motor pattern for feeding. Proboscis extension reflex (PER) is one of the well-known motor patterns in feeding behavior induced by stimulation of gustatory organs with phagostimulative tastants. PER is also controlled by the olfactory cues from the antennae. Generally, olfactory information is transmitted into the primary olfactory center, antennal lobe, in the brain. However, our behavioral experiments using the blowfly, Phormia regina, showed that a “favorite odorant (1.e., 1-octen-3-ol information)”, which increased their appetite, and an “aversive odorant (i.e., D-limonene)” information, which decrease their appetite, were detected by different sensory pathways from each other. On the other hand, the secondary olfactory organs, maxillary palps, are also moving during proboscis extension responses. Therefore, we investigated the role of the maxillary palps in feeding behavior. First, we tested whether the flies, when sufficiently starved, detect a favorite odor of 1-octen-3-ol by maxillary palps. The results showed that maxillary palps work as the detector of the appetitive odor enhancing feeding behavior. Next, we investigated whether the olfactory inputs from both sides enhance feeding behavior primarily prompted by stimulation of the labellar gustatory organ with sugar. The antennae ablated animals could not response to the aversive odor, while the maxillary palps ablated animals ignored the appetitive odor. Moreover, we found that either the left or the right neuronal pathways connecting between the olfactory inputs regardless of antennae or maxillary palps to the motor output as PER are independent of each other. In the present paper, we proved that the olfactory and the gustatory information are unilaterally integrated in the fly, and mentioned about a putative site, where such a unilateral integration occurs, in the brain.

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P 75

Mixture interactions in the taste sensilla of Drosophila melanogaster

The inhibition of sugar detection by antifeedants in the taste sensilla of insects is a phenomenon which has been known for more than 20 years. Dethier and Bowdan (1989; 1992) showed a decrease in the response to different sugars (glucose, fructose, sucrose) in presence of various alkaloids (quinine, berberine, caffeine) on Phormia regina, using the Proboscis Extension Reflex and electrophysiological recordings on the tarsi. However, the physiological mechanisms underlying this phenomenon are still unknown. Different hypotheses can be considered. Sugar inhibition could be mediated by receptors tuned to bitter molecules but located on the sugar cells and having an inhibitory activity. Or the bitter cell could have an inhibitory effect on the sugar cell through an unknown mechanism. We are trying to unravel part of the process involved in the interaction between sugars and antifeedants by studying this phenomenon on the proboscis taste sensilla of Drosophila melanogaster, using a combination of pharmacological and genetical approaches. References: Dethier VG, Bowdan E. 1989. The effect of alkaloids on sugar receptors and the feeding behaviour of the blowfly. Physiol. Entomol. 14: 127-136. Dethier VG, Bowdan E. 1992. Effects of alkaloids on feeding by phormia regina confirm the critical role of sensory inhibition. Physiol. Entomol. 17: 325-330.

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“Hasty tasting” of chemical information: functionally redundant peg sensilla in the scorpion pecten.

Scorpions sense their chemical environment with two mid-ventral appendages called pectines, which bear thousands of pore-tipped, peg-shaped sensilla on comblike “teeth.” Inside these pegs are chemosensitive neurons that detect volatile and aqueous stimulants. Limited research suggests that all sensilla have the same chemical sensitivity, a functional redundancy that may amplify sensory input (information enhancement hypothesis). An alternative hypothesis (information segmentation hypothesis) suggests that functionally different sensilla detect separate components of a complex stimulus. To test these hypotheses, we flooded the pectines of live scorpions (Paruroctonus utahensis) with mineral oil to create a medium for delivering water-soluble tastants. We simultaneously stimulated and recorded neurons by touching a sensillar pore with the tip of a glass pipette filled with 0.01 M KCl, 0.1 M citric acid (dissolved in 0.01 M KCl), or a 30% ethanol by volume solution (dissolved in 0.01 M KCl). The pipette also contained a silver wire to transmit signals to an amplifier and spike analysis program. This newly developed method is rapid and enables the investigator to stimulate one peg without affecting its neighbors. Moreover, this method detects the same active neurons as previously reported in recordings from peg bases. Each chemical elicited a unique response pattern among the sampled pegs (>270). Furthermore, when the data were analyzed by groups of pegs based on location within a tooth (distal, central, or proximal), all pegs appeared to respond similarly to the same stimulant. Taken together with the morphological similarity among sensilla, the brevity of a pectinal substrate “sniff” (0.033 s), and previous research with volatile odorants, our chemoresponse data support the information enhancement hypothesis. We argue that this arrangement provides for quick, accurate processing of chemical information, which may be of particular relevance to mate-tracking ability.

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Gustation in selachians: taste bud morphology, densities and distribution

Our studies describe the morphology, distribution and density of taste buds and papillae of the developing brown-banded bamboo shark, Chiloscyllium punctatum, and in mature specimens of Carcharhinus melanopterus, Hemiscyllium ocellatum, Negaprion acutidens, Orectolobus maculatus and Orectolobus ornatus. Morphology of taste buds appears comparable to that of teleost fishes, however, selachians appear to have very low densities of taste papillae ranging from as little as 4 per cm2 in the central oropharyngeal regions of O. maculatus to 165 per cm2 on the maxillary valve of C. melanopterus. The diameter of the taste papillae in C. punctatum increases with the size of the animal from 72 ± 1µm (embryo, 11.6cm TL) to 310 ± 7µm (mature individual, 110.3cm TL). C. punctatum maintains the same total number of taste papillae throughout life (mean = 1851±86) therefore, as the animal grows, taste papillae densities decrease as the oropharyngeal cavity increases in size. The highest densities of taste papillae in all species occur on the oral valves directly behind the teeth, which would enable maximal stimulation when prey is bitten. Densities throughout the rest of the oropharyngeal cavity appear constant. The densities of papillae in mature selachians are appreciably low in comparison to previous studies on teleost fishes, rats and mice used in other studies. This is probably due to their larger size since total numbers of taste papillae (ranging from 1354±127 in H. ocellatum to 11890±240 in N. acutidens) are comparable to other vertebrates.

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381 **P 78**

Stem and progenitor cell compartments within adult mouse taste buds

Adult taste buds are maintained by the lifelong proliferation of epithelial stem and progenitor cells, the identities of which have remained elusive. It has been proposed that these cells alternatively reside within the taste bud (intragemmal) or in the surrounding epithelium (perigemmal). Here, we apply three different in vivo approaches enabling single cell resolution of proliferative history to identify putative stem and progenitor cells associated with adult mouse taste buds. Experiments were performed across the circadian peak in oral epithelial proliferation (04:00 am), a time period in which mitotic activity in taste buds has not yet been detailed. Using double label pulse-chase experiments, we show that defined intragemmal cells (taste and basal) and perigemmal cells undergo rapid, sequential cell divisions and thus represent potential progenitor cells. Strikingly, mitotic activity was observed in taste cells previously thought to be postmitotic (labelled cells occur in 30% of palatal taste buds after one hour BrdU exposure). Basal cells showed expression of the transcription factor p63, required for maintaining the self-renewal potential of various epithelial stem cell types. Candidate taste stem cells were identified almost exclusively as basal cells using the label-retaining cell approach to localise slow-cycling cells (0.06 ± 0.01 cells/taste bud; n=436 taste buds). Together, these results indicate that both stem- and progenitor-like cells reside within the mammalian taste bud.

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Hormones, sex differences, social behaviors

593 **P 79**

Molecular Mechanisms for Environmental and Genetic Reversal of Sex-Biased Behavior

Differences between male and female behavior can be explained by differences in genetic makeup, sex hormones, and sexually dimorphic neuroanatomy, However, the expression of sex-typical behavior may also be regulated by the social environment. We use the African Julidochromis cichlid fish as a model to determine the underlying mechanisms that account for both differences in, and plasticity of, sex-typical behavioral phenotypes. Julidochromis, a genus of monogamous biparental cichlids, shows territorial and parental sex-typical behavioral roles. In some species, individuals exhibit "conventional" sex-biases in behavior such that the larger male provides territory defense while the smaller female provides nest care. Other species naturally pair in the reverse size ratio, and exhibit a reversal of behavioral roles. In both species, there is plasticity, such that behavioral patterns can be experimentally manipulated by controlling the relative size of the male and female in the pair. To examine the molecular changes accompanying plastic change in sex-typical behavior, we used microarrays to compare the gene expression of male and female brains under natural pairing and experimentally manipulated pairing. We studied J. transcriptus under both, male-larger pairs (natural-conventional) and female-larger (experimental-reversed), and we studied J. marlieri in female larger (natural-reversed) conditions. We find that the majority of gene regulation is both phenotype and species-specific in that there is little overlap between the gene lists for females of the two species, males of the two species, or either of the social phenotypes across sex and species. Interesting, the greatest intersection of gene lists is found for the aggressive phenotype suggesting that behavior, rather than sex, is the predominant determiner of gene expression profile.

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P 80

BEYOND THE INDIVIDUAL: SEX STEROID RECEPTORS MODULATE BEHAVIOR IN SOCIAL NETWORKS

Social networks can influence an individual's behavior and physiology, but little is known about how physiology and gene expression of one individual ripple through an entire community. We use the highly social fish, Astatotilapia burtoni, to study how social context influence behavior, hormones and neural gene expression across individuals within a community, focusing on androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR). We used pairs of dominant and subordinate males and recorded behavior, measured circulating hormones, and quantified sex steroid hormone receptor immunoreactivity and mRNA throughout the brain. We find robust covariance patterns within and between paired males, indicating that the behavioral, neuroendocrine and brain gene expression profiles of one individual profoundly affect (or are affected by) the respective profiles of another individual in the community. To infer the causal network relationships between sex steroid receptor expression, hormone levels and behavior across individuals, we treated dominant and subordinate males with AR, ER, and PR agonists and antagonists and examined hormone levels and behavior. We found that androgens and progestins regulate sexual behavior in dominant males while estrogen regulates aggression independent of social status. After treatment with antagonists, circulating hormone levels changed in subordinate males compared to controls. Finally, we measured preoptic gene expression of sex steroid receptors (and their subtypes), arginine vasotocin (AVT) and isotocin (IT) in these pharmacologically treated males, which allowed us to delineate the gene interaction network of sex steroid receptors in relation to physiology and social behavior.

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Is GnRH function mediated through an AVT receptor in a sex changing fish?

Arginine vasotocin (AVT) has been implicated in sex specific dominance and mating behaviors such as aggression, courtship, clasping and gamete release, while gonadotropin releasing hormone (GnRH) is a key hormone controlling reproductive development. Although, AVT is involved in behavioral sex change, GnRH has been implicated in gonadal sex change, and sexually dimorphic AVT and GnRH neuron populations have been identified in hermaphroditic fish. However no clear linkage has been identified between these two hormone systems. A study was initiated to investigate the linkage of AVT via a V1 type receptor (AVTr) and GnRH to behavioral and gonadal sex change in a small grouper the rock hind, Epinephelus adsenscionis. Rock hind is an appropriate model to study sexual inversion because of the unique male specific behavior and dramatic, temporary color patterning. It has a haremic reproductive strategy and removal of the dominant male causes the largest female to initiate sex change, with increased aggression observed in several days, and male specific color patterns evident in a matter of weeks. GnRH and AVT mRNA expressions determined by real-time quantitative PCR were higher in intersex fish than males or females. To map potential sites of action of AVT via its receptor in rock hind brain, an antibody for AVTr was developed. AVTr was widely distributed and highly expressed in brain areas associated with behavior and reproduction. Co-localization experiments revealed that AVTr was present on GnRH neurons. Ongoing research will track the changes in mRNA expression of GnRH, AVT, and AVTr during the period of behavioral sex change of the largest female by manipulating the social environment in captive rock hind social groups.

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P 82

Localizing the expression of *neuroserpin* in a teleost: Identifying brain regions associated with female mate preference

A female's choice of whom to mate with can have important individual and evolutionary consequences. While much is known about the neural mechanisms underlying female receptivity and pair-bond formation in rodents, relatively less is known about the mechanisms underlying the processes prior to copulation. Studies that examined female mate recognition and discrimination in birds and amphibians have used general neural activity markers (immediate early gene, IEG) to identify key auditory processing nuclei. Rather than use an IEG, here we use a female mate preference candidate gene, *neuroserpin*, (a serine protease inhibitor) to localize expression patterns associated with female mate preference in the teleost *Xiphophorus nigrensis*. In this study we localize and quantify the expression of *neuroserpin* to identify brain regions that may underlie female mate preference behavior in wild caught *X. nigrensis*. Forty females were subjected to one of several conditions in a non-contact dichotomous choice setup for 30 minutes. We recorded the association time and two behaviors exhibited during the trial to assign a preference score. Using *in situ* hybridization we quantified the expression of *neuroserpin* in ten nuclei. We demonstrate that three forebrain nuclei show a significant difference in *neuroserpin* expression between "high" and "low" preference score females only in mate choice conditions. Furthermore, significant positive relationships between *neuroserpin* expression and preference score were observed in Dm, Dl and POA. Interestingly, there is a unique *neuroserpin* expression network between the nuclei examined that is seen only in mate choice conditions. This study identifies nuclei previously not implicated in mate recognition/discrimination studies and the brain may have a characteristic *neuroserpin* expression pattern across several nuclei during mate choice.

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653 **P 83**

The social behavior network in anamniote vertebrates: weakly pulse-type electric fish and hylid anuran.

Vertebrates exhibit different social behaviors that are regulated by an evolutionarily conserved, core social behavior network (SBN) of the forebrain and midbrain. Steroid hormones (androgens, in particular) and hypothalamic neuropeptides (AVT, in particular) participate in the activation of these behaviors acting on brain regions that belong to the SBN. We selected two related species with different social organization (Brachyhypopomus gauderio and Gymnotus omarorum) and two non-related species with similar social organization (B. gauderio and Hypsiboas pulchellus) to study the neural bases of social behavior. G. omarorum and B. gauderio are weakly pulse-type electric fish. G. omarorum is solitary and highly aggressive whereas B. gauderio is a gregarious species that only displays seasonal aggressive behavior. H. pulchellus is a social hylid anuran. During the courtship behavior of both gregarious species, males emit conspicuous signals: electric chirps in B. gauderio and vocalizations in H. pulchellus. We focused our exploration on one region of the SBN: the preoptic area (POA) of the hypothalamus. We searched for neuroanatomical differences and similitaries in the POA that could correlate with the properties of the social organization in these vertebrate species. The neuroanatomical characterization of the POA was done for the first time in this study. AVT neurons were immunodetected in the POA of the three species, and we observed a difference in the pattern of distribution of AVT neurons between G. omarorum and B. gauderio. AR expression detected by immunohistochemistry, using PG-21 antibody, was found in different nuclei of the POA in the three species. Neuronal activity in the POA in response to social stimuli was preliminary measured by immunodetection of early gene expression protein (c-Fos) in B. gauderio.

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Gender-specific differences in echoacoustic orientation in the lesser spear-nosed bat (Phyllostomus discolor).

The hippocampal formation is widely accepted to play an important role in allocentric spatial cognition in many, if not all, mammals. Anatomical differences of this brain area between males and females are believed to correspond directly to gender-specific differences in orientational strategies that were found in several mammalian species, with males preferentially using geometric cues when it comes to orientation, whereas females predominantly use the information from landmarks. Most studies investigating into the nature of gender-specific orientation have focused on the role of visual landmarks, but due to the highly multi-modal input the hippocampal formation receives, it is very likely that the same phenomenon exists for other modalities, too. To test this hypothesis, we used the echolocating bat Phyllostomus discolor (lesser spear-nosed bat) for a behavioural study on gender differences in the use of acoustic landmarks. Based on three lines of evidence, we are the first to show that such gender differences in the use of acoustic landmarks exist and conclude that the phenomenon in general is independent of the modality that is used to sense the environment during orientation. Our findings therefore allow for the prediction of similar phenomena in other acoustically orienting mammals, including humans (especially the blind). Interestingly, due to the specific ecology of P. discolor, our results partially contradict the evolutionary theories on gender-specific orientation, as will be discussed. Finally, we consider our finding as being an important step towards establishing microbats as a new model organism in neuroscientific studies on allocentric spatial cognition in mammals.

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EVOLUTION OF NEURAL NETWORKS REGULATING SOCIAL BEHAVIOR

Little is known about the evolution of the neurocircuitry that mediates important behavioral decisions, such as mate choice and social dominance behaviors. Two (partially overlapping) neural networks that seem to regulate social decision-making are the midbrain dopaminergic reward system and Newman's social behavior network. We propose that brain nuclei in these circuits are part of a social decision-making network that provides a powerful framework for understanding the evolution of social behavior and its underlying neural circuitry. We have compared neurochemical profiles of brain regions that compose the dopaminergic reward system and social behavior network across teleosts, amphibians, reptiles, birds and mammals. In this comparative analysis, we have evaluated eight neuroendocrine (sex steroid hormone receptors, neuropeptides, and neuropeptide receptors) and dopaminergic genes (tyrosine hydroxylase and dopamine D1 receptor) across the 13 brain regions encompassing this large neural network. We find a surprising degree of conservation across vertebrate classes, indicating that there is an evolutionary conserved core-SDM network already present in early vertebrates. We have found the neurochemistry of some brain regions is variable across vertebrates, but other regions are highly conserved. Throughout the whole network, we have found that where there is variation, mammals and fish are most similar given these gene expression profiles. We find that variation across the major vertebrate classes occurs with the distribution of "upstream" signaling elements, the neuropeptide- and dopamine-producing cells, whereas the distribution of "downsteam" elements, such as receptors, are very conserved. We speculate that sensory integration can vary in a species-specific manner, whereas the socially mediated decision making mechanisms have been in place for >500 million years of vertebrate evolution. Thus, the SDM network likely arose from an evolutionarily ancient core network of brain nuclei.

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Influence of early song memory on differential reproductive allocation in Bengalese finch females

Female preference plays a key role for song evolution in oscine species. In order to look into the social mechanisms underlying the development of song preference, we examined the effect of early social environment on female reproductive behaviors in adulthood. In many vertebrate species, young individuals learn the characteristics of a desirable mate through the learning process of sexual imprinting, which might cause inbreeding in some cases. Indeed, it has been reported that female songbirds tended to prefer father-like songs. According to differential allocation theory, females are expected to adjust their reproductive investment depending on the attractiveness of mates. So, we tested if females invested more when mated to males whose songs sounded □gfamiliar□h. Subject females of the Bengalese finch had been reared by foster parents until fledging period when a subtutor (unrelated adult male) was additionally introduced into each breeding cage, to simulate natural social environment in which chicks have more opportunity to interact unrelated adult individuals after fledging. We sequentially paired these females to two different males and investigated if the differences in their reproductive behaviors between two breeding episodes were affected by the song traits of their mates. We found that song similarity between the foster father and mates had no effect. However, females laid heavier eggs when mated to males whose songs were acoustically more similar to those of the subtutor. These results suggest that female song preference was subject to early song memory. We can assume that complex songs (larger repertoire or higher versatility) should be favored when social environment during breeding is not monotonous and females have diverse early song memories.

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Neuro-economics in chicks: competitive foraging socially increases work investment and chronically enhances impulsive choices in domestic chicks.

Animals (including humans) often choose an immediate reward even when a delayed alternative option yields a larger gain. Since this behavioral trait clearly characterizes impulsiveness measured in terms of the temporal discounting, relevant neural and developmental factors have so far been intensively studied (Cardinal 2006, Kalenscher & Pennarts 2008, Matsushima et al. 2008, Trip & Wickens 2008). It has been suggested that the discounting occurs because when there is a longer delay, there is a higher risk being interrupted and consequently losing the reward, i.e., □gcollection risk hypothesis□h (McNamara & Houston 1987, Benson & Stephens 1996, Sozou 1998). Although this hypothesis is intuitively simple and plausible, the functional link between competition and impulsiveness has not been empirically supported. Here, in a series of behavioral experiments, we examined social factors involved in the control of impulsiveness and work investment in domestic chicks. In binary choices between a large / long-delay option (LL) and a small / short-delay alternative (SS), chicks that had been competitively trained in group of 3 individuals showed fewer choices of LL than non-competitive controls, even when tested 2 days after the end of training. Such a chronic effect occurred when chicks were separated through a transparent window, indicating that the actual loss of gain nor the delay-proportionate interruption was not involved. Rather, perceived competition was sufficient in the enhanced impulsiveness. The social competition also acutely increased the work investment measured as the total locomotor activities in I-shaped maze, each terminal of which was equipped with a low-profitability feeder of variable intervals. Localized lesion experiments and single unit recording in freely behaving condition revealed striatal and pallial (isocortical) structures controlling the impulsiveness and work investment. Effects of social competition on these neural substrates will be discussed.

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P 88

Housing conditions affect neural activity and functionnal connectivity within the social behavior network in male zebra finches (Taeniopygia guttata)

The "social behavior network" (SBN) is a set of limbic regions involved in many social behaviors in vertebrates. Its activity has generally been studied during short encounter tests, in which one individual is subjected to various social stimuli like the presentation of an intruder or a sexual partner. But, long-term social and physical environment could also induce neural changes. In particular, the housing conditions previously experienced by experimental animals could solely modify the neural substrates. Physical isolation in individual cages represents an impoverished environment on both physical and social aspects in gregarious animals. It prevents animals from socializing even when auditory and visual contact is maintained. In this study, we compared the activity and the functional connectivity of the septo-hypothalamic regions of the SBN between male zebra finches (Taeniopygia guttata) housed in individual cages and communally housed males who freely and repeatedly interacted in social group. The activity of four structures of the SBN (BSTm, bed nucleus of the stria terminalis; POM, medial preoptic area; lateral septum; ventro-medial hypothalamus) and one associated region (paraventricular nucleus of the hypothalamus) was assessed using immunoreactive nuclei density of the immediate early gene Zenk (egr-1), and was related to behavioral activities of birds like physical and vocal interactions. We further assessed the identity of active cell populations by labeling vasotocin (AVT). We showed that housing this gregarious species in individual cages increases Zenk labeling in non-AVT cells of the BSTm and enhances the correlation of Zenk-revealed activity between the studied structures. Moreover, the rate of social interactions (aggressive vs affiliative) of birds in social group is correlated with both testosterone levels and density of labeled cells in the SBN.

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Functional Roles for ETH Signaling in Adult Drosophila melanogaster

Ecdysis triggering hormone (ETH) is an endocrine peptide regulator of sequential behaviors underlying ecdysis in all insects examined. Because ETH expression in endocrine Inka cells persists following eclosion, we have been interested in identifying its possible functional roles in adults. Recent evidence indicates that ETH receptors (ETHRs) are expressed in adult corpora allata (CA), the sole source of juvenile hormone (JH). JH is known to have numerous gonadotropic and behavioral functions associated with reproduction. We found that exposure of CA to ETH leads to robust calcium mobilization in both males and females. RNAi silencing of ETHRs in the CA leads to altered courtship behavior. Our data indicate that physiological functions of ETH extend beyond ecdysis regulation in immature stages to include adult reproductive behaviors.

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A role of hugin signaling in the innate food preference behavior of Drosophila melanogaster

Feeding is an essential innate behavior which allows animals to maintain optimum nutrimental states for the growth and metabolism. Fruit fly Drosophila melanogaster is a genetically tractable model organism for the study of neuronal mechanisms underlying diverse innate behaviors. Like other animals, Drosophila chooses between different food sources depending on its physiological needs. In the laboratory condition, adult flies show sexual state-specific food preference behaviors. When given with a choice between sucrose and yeast, both males and virgin females show strong preference toward sucrose. On the contrary, mated females prefer yeast to sucrose. Diverse aspects of Drosophila feeding behaviors were shown regulated by brain-born neuropeptides, such as neuropeptide F, short neuropeptide F, insulin-like peptides and hugin. Especially, hugin-producing neurons have been implicated in food intake initiation. In this study we investigate roles of hugin signaling in the innate food preference. To test if hugin neurons regulate the food preference, we silenced hugin neurons by expressing an inward rectifying potassium channel, Kir2.1. Virgin females with silenced hugin neuron (hugin-KO) showed the strong preference toward yeast, which is normally observed in mated females. In contrast, control lacking Kir2.1 expression and wild type virgins showed the preference toward sucrose. This phenotype of hugin-KO was also observed in null mutants of a hugin receptor gene, CG8795. These results suggest that hugin signaling might shape the sexual state-specific innate food preference in Drosophila.

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P 91

Functional dissection of the neural circuits regulating aggression in Drosophila melanogaster

Similar to most animals, Drosophila melanogaster exhibit an innate capacity for aggressive behavior that enables them to compete for food, mates and territory. Males will engage in a set of stereotyped agonistic interactions until a dominance hierarchy is established. Aggressive behavior in vertebrates and invertebrates is strongly influenced by neuromodulatory systems, as well as environmental factors such as past social experience with conspecifics. However, the neural circuits that mediate aggressive behavior, and where and how in this circuitry factor that influence aggression act, remain largely unknown. To identify neural circuits involved in aggressive behavior, we have developed a high-throughput, automated assay of Drosophila male social behavior that uses machine vision software (CADABRA; Dankert et al., Nat Methods 2009) to quantify aggressive and courtship interactions between pairs of males. Using this system, we are conducting a large-scale GAL4 screen to identify neurons that promote aggression when transiently activated using the temperature-sensitive Drosophila TRPA1 channel. We have identified several lines that show a robust, temperature-dependent, increase in aggression. A detailed analysis of these phenotypes shows an enhancement in both the frequency of aggressive interactions and the escalation to higher intensity aggressive behaviors. Lastly, we describe the results of secondary behavioral assays aimed at assessing the specificity of the neuronal activation phenotype to aggression; determining the necessity of the neurons for aggression; and assessing how the activation of these circuits influences the expression of aggression in different social contexts.

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634

P 92

Unattractive males can sire attractive sons in Drosophila melanogaster

In Drosophila simulans, male attractiveness is heritable through the patriline: attractive fathers sire attractive sons, while unattractive fathers sire unattractive sons (Taylor et al., 2007). We tested if this rule also holds for Drosophila melanogaster. We took advantage of recently published 40 isogenic lines with considerable variability in copulation latencies and frequencies. We started by measuring copulation latencies and frequencies of a selected subset of these lines in standard courtship wheels against a Canton S tester female. The selected lines were the five lines with the highest (lowest, respectively) copulation latencies from experiments using large, food-containing vials with Oregon/Samarkand tester females. To identify the lines best suited for beck-crossing, the copulation latencies were plotted against the copulation frequencies and the two lines most clearly separable on both variables were chosen for crossbreeding. The male offspring from both of the reciprocal crosses were similar in both latency and frequency to the more attractive line, consistent with an autosomal dominant heritability of attractiveness in D. melanogaster. This evidence demonstrates that the fathers from an unattractive D. melanogaster population can sire attractive sons if they mate with females from a population that produces attractive males. These results confirm and extend previous findings that the two closely related Drosophila species contrast dramatically in the effect of sexual selection on the heritability of attractiveness. Reference: Taylor ML, Wedell N, Hosken DJ. (2007): The heritability of attractiveness. Taylor ML, Wedell N, Hosken DJ. Curr Biol. 17(22):R959-60

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Motor Systems

669 **P 93**

Symmetric and asymmetric breathing in flatfishes (Pleuronectiformes)

Alexander Bublitz, Benedikt Niesterok and Wolf Hanke Sensory and Cognitive Ecology, Institute for Biosciences, Rostock University, Albert-Einstein-Strasse 3, 18059 Rostock, Germany; wolf.hanke@uni-rostock.de. Breathing currents of fish, that is the stream of water expelled from the gill openings, constitute a potential source of information for piscivorous predators that use hydrodynamic sensory systems to detect prey. We investigated the breathing currents of three different teleost species using Particle Image Velocimetry (PIV). One fish at a time was positioned in a tank (80*35*40 cm). The water in the tank was seeded with neutrally buoyant particles and illuminated with a laser light sheet generated with a 500 mW DPSS laser. Pictures of the illuminated particles were recorded with an Allied Vision Technologies Pike F032C camera at 12 to 50 frames per second. Most surprising was the strong breathing current found in flounders (Platichthys flesus) that were lying on or buried in the substrate. In terms of water velocity, breathing currents in flounders were 4 times stronger than in eel (Anguilla anguilla) and 11 times stronger than in rainbow trout (Oncorhynchus mykiss). We suspected that asymmetric breathing in the flatfishes might be responsible for part of these differences. However, earlier studies on breathing in flatfishes using cineradiographic recordings, electromyographic recordings, and recordings of pressure changes in the respiratory cavities, claimed to have unequivocally shown that flatfishes, even lying on or buried in the substrate, generally breathe symmetrically. According to this theory they would have to create a cavity between the blind side and the ground using their fins. Investigating the breathing currents in flatfishes that were suspended in a wide mesh wire cage in midwater, we found that both symmetric and asymmetric breathing occur. This explains the presence of an interconnecting channel between the opercular cavities, the role of which would otherwise be obscure. We suggest that flatfishes can switch from symmetrical to asymmetrical breathing. The factors that trigger this switch are being investigated. Supported by the German Research Foundation (DFG) (SPP1207)

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P 94

The energy expenditure of trout swimming in laminar and turbulent flow

Rheophilic fish like trout are constantly exposed to running water and flow perturbations. Instead of preferring the undisturbed flow regions, trout often hold station in perturbated flow zones. For instance, in a flow tank trout use the vortex street caused by a submerged cylinder to save energy, i.e. by Kármán gaiting (Liao et al., J Exp Biol 206, 2003). In addition trout may swim in the bow wake zone or next to the side and slightly downstream of the cylinder (entraining). Liao et al. (Science, 302, 2003) compared the muscle activity of Kármán gaiting and freely swimming trout. They found that Kármán gaiting trout need less energy for station holding. Since Liao et al. (J Exp Biol 207, 2004) analyzed only short (20 tailbeat cycles) periods of time, the total energy expenditure of trout swimming in the Kármán gait zone cannot be predicted. To our knowledge the muscle activity of entraining trout or of trout swimming in the bow wake zone has never been measured. Therefore we studied the swimming behavior of individual trout (N = 5) for three hours in a flow tank (bulk flow velocity 20, 28, 37, and 49 cm/s) that contained a submerged cylinder while continuously recording their swimming muscle activity. We confirm that the bow wake, entraining, and Kármán gaiting zone are the regions preferred by trout. The mean muscle activity in all of these regions is lower than that of trout swimming in undisturbed flow. A fourth hitherto undescribed region preferred by trout is close to the water surface behind the cylinder. Supported by the DFG

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Developmental plasticity of spinal efference copy signaling in extraocular motoneurons during locomotion in metamorphosing Xenopus frogs.

In Xenopus laevis tadpoles, retinal image stabilization during locomotion not only relies on visuo- and vestibulo-ocular reflexes, but is also partly achieved by efference copies of spinal locomotory CPG commands that produce rhythmic extraocular motor activity appropriate for eliciting conjugate, compensatory movements of the eyes. During metamorphosis, Xenopus switches its mode of locomotion from larval tail-based undulatory movements to bilaterally-synchronous hindlimb kick propulsion in the adult, with intervening stages where functional larval and adult locomotory systems co-exist within the same animal. The change in locomotory mode leads to body motion dynamics in adult frogs that require particular, non-conjugate eye motion patterns for adequate retinal image stabilization. We have investigated spino-extraocular motor coupling during this developmental transition in order to understand how and when gaze control processes are altered in accordance with the animal's change in body plan and movement strategy. Simultaneous recordings of different extraocular, limb (extensor and flexor) and tail motor nerves during spontaneous fictive swimming were performed on brainstem/spinal cord preparations from different metamorphic stages. Our results show that there is indeed an associated change in spinal efference copy control of extraocular motor output. For example, in contrast to fictive axial swimming which drives alternating bursts in bilateral medial rectus (MR) nerves that in vivo would produce eye movements in opposite direction to angular head excursions, during fictive limb extensions, the MR motor nerves are synchronously active in correspondence with a need for convergent eye movements during the linear head accelerations produced by forward propulsion. Thus, neural plasticity during metamorphosis allows spinal CPG-derived extraocular motor activity to be functionally adapted to the respective spatial and dynamic requirements for compensatory eye motion.

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Muscarinic Actions in Xenopus laevis Tadpole Swimming

The neurotransmitter acetylcholine (ACh) acts on nicotinic and muscarinic receptors (mAChRs). In the central pattern generator (CPG) controlling Xenopus laevis tadpole swimming ACh has been shown to be released from both motor neurons and descending interneurons. Atropine, a specific mAChR antagonist, has been shown to reduce swimming duration but the cellular mechanisms involved are unclear. We aim to re-assess muscarinic actions in tadpole swimming and how these change in early Xenopus development. Standard extracellular recordings were made from ventral roots of immobilised Xenopus laevis tadpoles to measure fictive swimming. Preliminary experiments identify two effects of the mAChR agonist carbachol (0.3-100ìM) on fictive swimming (n=25 tadpoles). 1) Threshold for skin stimulation to initiate fictive swimming was increased across the concentration range. Concentrations above 15µM abolished the response to skin stimulation. Light dimming could still induce swimming implying that the effects are likely to be a result of modulation of the mechanosensory pathway. 2) When carbachol was applied at concentrations above 3µM there was an increase in swimming activity. Spontaneous swimming occurred more often and swimming episode duration tended to increase. These results indicate an increase in the excitability of the swimming CPG. The effects described in 1) and 2) were seen in spinalised animals (n=2) suggesting the swimming circuit is a direct target of carbachol. 10µM atropine decreased swimming episode duration in both stage 37/38 (n=6) and 42 (n=5) tadpoles. A decrease in stimulation threshold was only seen at stage 42. This indicates a difference in the onset of mAChR actions during development between the sensory pathway and the swimming CPG. Whole cell recordings will be carried out to identify the cellular mechanisms that mediate these muscarinic actions.

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Nitrergic neuromodulation of locomotor activities in Xenopus laevis tadpole

The spinal central pattern generator (CPG) for swimming in Xenopus tadpoles has been well explored, providing an excellent model system for investigating the modulation of vertebrate locomotor circuitry. It has been shown that the endogenous release of nitric oxide (NO) from groups of brainstem neurons decreases swimming episode duration and cycle frequency by facilitating glycinergic and GABAergic inhibition (Mclean & Sillar, 2002). However the modulatory effects of NO at the cellular level, on spinal neurons, are largely unknown. In the present study we investigated the effect of NO on the activity of individual spinal neurons in vivo by using current-clamp patch recordings while monitoring the ventral root activity of Xenopus tadpoles (Stage 37/38 and 42). NO donors, SNAP (200 micromolar) or DEA/NO (200 micromolar), both shortened the swimming episode duration and slowed down the swimming frequency, consistent with previous studies. On the cellular level, SNAP and DEA/NO depolarized the resting membrane potential of spinal neurons by up to 5mV and a small but clear hyperpolarization appeared after washed NO donors away, effects which still existed in the presence of 1 micromolar TTX. The amplitude of action potentials and the firing probability during swimming were reduced. Spinal neurons also showed stronger accommodation during step depolarizing pulses after NO donor application. The specific NO scavenger C-PTIO (200 micromolar) abolished the effect of NO donors. To test the endogenous NO effect we provided novel NOS substrate AKO and confirmed that enhanced endogenous NO release had a similar effect to the NO donors. Directly enhancing the NO level in saline by adding nitrite (2mM) also reduced the swim episode duration and swim frequency. Our results suggest that NO is involved in the neuromodulatory control of the duration and intensity of swimming activity by regulating the properties of spinal CPG neurons. We are grateful to Dr. N. Botting, School of Chemistry, University of St. Andrews, for providing AKO.

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P 98

Hindbrain neurones and the initiation of swimming - mapping responses to head sensory stimulation in hatchling tadpoles of Xenopus laevis

In most vertebrates the exact neuronal pathway responsible for the initiation of locomotion after sensory stimulation remains unclear. Hatchling tadpoles start swimming some 20-30 ms after their head skin is touched. This touch excites trigeminal sensory neurones projecting into the hindbrain. These neurones must activate some currently unknown sensory pathway interneurones integrating and distributing the information. These in turn should activate the identified population of excitatory dIN reticulospinal neurones which are thought to generate the swimming rhythm and drive spinal neurone firing during swimming (Soffe et al., 2009). We map the responses of hindbrain neurones to skin stimulation by using whole-cell patch recordings under visual control in intact immobilised tadpoles while recording from ventral roots to monitor motor responses. By filling the cells with neurobiotin, we can define their morphology after the experiment. We found a range of neurones which were excited and spiked to head skin stimulation. Some were centrally located primary sensory neurones, spiking some 4 ms after the stimulus. Other neurones were on the stimulated side (including dINs), and on the contralateral side. They fired over a continuous range of longer latencies. Some neurones also received short latency inhibition indicating that inhibitory interneurones were directly excited by trigeminal sensory afferents. We hope to specify the pathway and understand the neurones involved in the swim initiation pathway and the decision to swim. This could lead to a general understanding of the mechanisms for the initiation of locomotory behaviour which still remain largely unclear.
Soffe SR, Roberts A, Li WC (2009) Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control. J Physiol 587:4829-4844.

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How hatchling Xenopus tadpoles avoid being sucked up by predators

Newly hatched Xenopus tadpoles, like any small pelagic animal, can be predated by fish and also by older metamorphosed amphibians which feed by sucking prey into their mouths. Cannibalism by adults of their own species is well documented. We ask whether young tadpoles have any defence against such attack. To model suction feeding, we sucked tadpoles quickly into a glass tube and observed that they frequently swam straight out again. This "swim-out" response was first seen before normal hatching at stage 33/34 and increased in reliability after hatching to stage 40. We have recently shown (Roberts et al., 2009) that the lateral-line sensory system of the tadpole becomes functional around the stage of hatching. We found that the "swim-out" response depends on the presence of lateral-line neuromasts in the post-orbital region since it was blocked by lesions to these organs and by neomycin treatment. To observe the responses of tadpoles sucked into a glass tube we used a Casio EX-F1 camera to record high-speed videos. The videos (at 300 fps) showed that, regardless of their orientation on entering the pipette, tadpoles could turn within 100 ms and then swim out against initial direction of the water flow. Older tadpoles could bend more and swim-out more reliably. The CNS circuitry that allows such a simple animal to have such adaptive behaviour is currently under investigation. REFERENCE: Roberts, A., Feetham, B., Pajak, M. and Teare, T. (2009) Responses of hatchling Xenopus tadpoles to water currents: first function of lateral line receptors without cupulae. J exp Biol 212: 914 - 921

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P 100

Intrinsic self modulation: reticulospinal neurons switch each other into pacemaker mode to drive movement

Defining the source and mechanisms of rhythmic neuronal network activity is a major challenge. In the vertebrate brain, rhythms for breathing and locomotion usually rely on circuits of neurons involving inhibition combined with neurons acting as pacemakers. By studying a simple system, the hatchling Xenopus tadpole, we have identified a population of glutamatergic reticulospinal and spinal neurons (dINs) which drive swimming locomotion. These dINs fire a single spike to current, are electrically coupled, and make mutual synapses with each other activating NMDARs. During swimming dINs fire once per cycle to drive spinal CPG neurons. We have concluded that one mode of swimming rhythm generation is network based and depends dINs firing on rebound following reciprocal inhibition. However, a hemi-CNS with inhibition blocked pharmacologically can still generate swimming-like rhythms. We show here that activation of NMDARs can transform dINs from firing singly into pacemakers firing within the normal swimming frequency range (10-25 Hz). When TTX blocks dIN firing, NMDA produces 10 Hz membrane potential oscillations which persist when electrical coupling is blocked but does not occur when Mg2+ is removed from the saline. The NMDA induced 10 Hz oscillations and swimming frequency pacemaker firing are unique to the dIN population and do not occur in other neurons. We conclude that NMDAR mediated self-modulation switches dINs into pacemaker mode only during swimming where it provides an additional, parallel mechanism for locomotor rhythm generation. Such conditional pacemaker properties are an emerging feature of rhythm generation in many regions of the vertebrate brain.

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P 101

Roles of identified interneurons in the mouse spinal locomotor central pattern generator

The search to understand the organization of the Central Pattern Generator network that drives locomotion in the mouse spinal cord has been simplified by the generation of transgenic mice expressing GFP in specific interneuron classes identified by selective transcription factor expression. Here we report on our studies of two such classes using electrophysiological and calcium imaging methods. The Hb9 interneurons (INs) have oscillatory properties and rhythmic activity during fictive locomotion, and have been suggested to participate in the rhythm-generating network of the locomotor CPG. We have explored the timing of activity of these neurons during fictive locomotion, using calcium imaging and whole cell recording. Bouts of fictive locomotion can be evoked by tonic stimulation of the caudal spinal cord. Hb9 INs are rarely active before the first onset of ventral root activity; rather, most become active well after the onset of motoneuron bursting. During subsequent cycles of locomotor-like activity, the Hb9 IN onset phase continues to lag behind the onset of ipsilateral ventral root bursts. In addition, most Hb9 INs stop firing after several cycles, while rhythmic ventral root bursts continue. Similar results are seen with transmitter-evoked fictive locomotion: the Hb9 interneurons do not fire on every cycle, and when they do, their onset phase lags behind the onset of ipsilateral ventral root activity. Thus, although the Hb9 INs are rhythmically active and could participate in the locomotor CPG, they do not fire at the correct phase to provide the major intrasegmental rhythmic drive for the locomotor network. Second, the ipsilaterally projecting glutamatergic V2a INs can be identified by their Chx10 expression. They are rhythmically active during fictive locomotion, firing in phase with either the ipsilateral flexor or extensor ventral root bursts, and synapse on commissural interneurons to regulate left-right alternation. When these neurons are deleted, in Chx10-DTA mice, a speed-dependent locomotor phenotype appears. Intact Chx10-DTA mice run normally at low speeds, show disrupted locomotion at intermediate speeds and switch to a synchronous “galloping” gait at high speeds, which is never seen in wild type mice. This speed-dependent gait shift from left-right alternation to left-right synchrony is also seen during fictive locomotion in the isolated Chx10-DTA spinal cord. In normal spinal cords, the V2a INs show increased activity and increased rhythmicity with increased locomotor speed in the isolated spinal cord. These results suggest that the V2a INs normally drive commissural pathways to maintain left-right alternation, but only at high speeds; other neurons must drive left-right alternation at low speeds. Supported by the NSF (0749467) and the Christopher and Dana Reeve Foundation.

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P 102

Regional distributions of active spinal cord neurons during fictive forward swimming and three forms of fictive scratching

Research has suggested that animals can utilize both shared and specialized circuitry to generate different behaviors involving the same muscles (Berkowitz et al., 2010). In the turtle spinal cord, examples of both types of circuitry have been identified in the control of three forms of scratching and forward swimming (Berkowitz, 2002, 2008). Little is known, however, about the anatomical distributions of neurons active during these different behaviors. The regional distributions of active neurons during fictive swimming and three forms of fictive scratching were previously examined in a series of horizontal spinal cord sections (Willis and Berkowitz, 2008). Here we examine the cross-sectional distribution of active neurons in transverse sections. Spinalized and immobilized turtles were stimulated repeatedly to produce one form of fictive motor pattern for 3-12 hours. Active neurons were labeled with sulforhodamine 101, a fluorescent activity-dependent dye (Keifer et al., 1992). Following perfusion, spinal cord segments including the five segments of the hindlimb enlargement (D8-S2) and two pre-enlargement segments (D6-D7) were removed, sectioned and photographed for analysis. Results show that labeled neurons were distributed across the examined segments in all animals, both ipsilateral and contralateral to the fictive limb movement. However, active neurons were concentrated in anterior segments (D7-D9) in all animals. In addition, more labeling was seen ipsilateral to the fictive limb movement in most but not all animals. Following fictive swimming, most labeling was in the dorsal horn bilaterally and in the ipsilateral intermediate zone and dorsal part of the ventral horn. In contrast, following fictive scratching, labeling was more distributed and also included the ventral part of the ventral horn bilaterally. The regions of labeling unique to one motor pattern may indicate concentrations of specialized neurons. Supported by NSF grants IOB-0349620 and IOS-0950370 to A.B.

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P 103

Strong interactions between the networks for swimming and each form of scratching in turtles

Different behaviors involving a common set of muscles can be generated by separate networks (Ramirez and Pearson 1988, Hennig 1990), a single network (Marder and Calabrese 1996), or partly overlapping networks (Berkowitz et. al. 2010). Overlap or interactions between networks can be revealed by simultaneous activation of two networks. The turtle spinal cord contains networks for locomotion (forward swimming) and three forms of scratching (rostral, pocket, and caudal) (Stein 2005). Simultaneous activation of the networks for rostral scratching and forward swimming, which share a knee-hip synergy, can produce hybrid movements (Earhart and Stein, 2000). Fictive rostral scratching and forward swimming can each interrupt and reset the rhythm of the other (Juranek and Currie 2000). We now show that the network for each form of scratching can interact with the network for forward swimming to modify motor patterns. In immobilized, spinal turtles, we evoked each form of fictive scratching by mechanical stimulation of a site on the ipsilateral body surface and fictive forward swimming by electrical stimulation of the contralateral lateral funiculus. We found that combining swim stimulation with each form of scratch stimulation could 1) alter the amplitude, phase, and/or duty cycle of motor nerve bursts, 2) increase the rhythm frequency, 3) establish a normal swim motor pattern even if the swim stimulation alone was too weak or too strong, and 4) disrupt rhythm generation entirely. The current results extend previous findings by showing that pocket scratching and caudal scratching, which do not share a similar knee-hip synergy with forward swimming, can also interact with swim stimulation. The findings of increased rhythm frequency in some cases of combined stimulation and rhythm disruption in other cases may also suggest that swimming and scratching inputs converge prior to rhythm generation. Supported by NSF grants IOB-0349620 and IOS-0950370 to A.B.

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From single neuron to intact animal: Studying intersegmental coordination for crawling in the medicinal leech

Descending signals from the brain are known to be important for controlling locomotion in animals that express flexible forms of locomotion such as walking. Previous work in our lab using the medicinal leech has established that descending signals from the cephalic ganglion (i.e., brain) are both necessary and sufficient for crawling behavior and normal intersegmental coordination (Puhl and Mesce, 2010, J. Neurosci. 30:2373-2383). In this study we aimed to: 1) characterize individual long-distance-projecting brain neurons that may play a role in the CNS-wide activation and intersegmental coordination of the crawl oscillators, and 2) investigate the behavioral ramifications of transecting the CNS in intact leeches. We studied a bilaterally-paired descending brain neuron, R3b-1 (Esch et al., 2002, J. Neurosci. 22:11045-11054), which gates fictive crawling and swimming behaviors in isolated CNS preparations. In the presence of exogenous dopamine (DA), however, intracellular stimulation of R3b-1 led exclusively to fictive crawling. Exposure to DA also induced rhythmic oscillations in R3b-1, which were often phase-locked to crawl motor bursts. At times when DA initiated only uncoordinated crawl-like activity, R3b-1 intracellular excitation caused intersegmental coordination. Furthermore, electrical stimulation of R3b-1 during coordinated DA-induced fictive crawling led to perturbations in the intersegmental propagation and frequency of the crawl motor pattern. These results provide evidence that this single neuron is a critical component in the brain's influence over crawling behavior. In a series of complementary experiments, we completely transected the nerve cords of intact animals between the brain and first segmental ganglion (M1), which led to an immediate loss of overt crawling behavior. Leeches retained their ability to swim, however, and expressed reflexes such as shortening and local bending. Remarkably, ca. 10-14 days after transection, these animals began to express crawl-like movements without any observable regeneration of their nerve cord. Collectively, these data suggest that in the normal condition descending signals from the brain, such as those from R3b-1, are vital for crawling. In their absence, the segmental crawl oscillators appear to become reorganized and susceptible to novel inputs that aid in their activation and coordination.

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571 **P 105**

Fictive flirting: a new behavioral pattern generator

In a broad range of species, sexually naÃ¯ve organisms perform reproductive behaviors, which suggests that in these species reproductive behavioral programs are hard-wired in the nervous system, rather than learned. Hormonal signaling has long been known to modulate these reproductive behaviors, but how hormones change neuronal activity has been challenging to study. We are investigating how hormones in the vasopressin family produce reproductive behavior in Hirudo verbana. We have used (Arg8)-conopressin G, initially isolated from the venom of the cone snail Conus imperialis; Hirudotocin, which has been found in Hirudo; and annetocin, discovered in earthworms. All three peptides produce highly stereotyped courtship behaviors: injecting any of the three at ÂµM concentrations shifted a leech''s behavior into the reproductive repertoire, but Hirudotocin and Conopressin produced these behaviors at a lower dosage compared to annetocin. What is the native source of the hormone in leeches? Previous immunohistological work identified leech neurons that stained positively for mammalian vasopressin. We have repeated those experiments and found in many ganglia along the nerve cord a small population of neurons that reliably stained when we probed for vasopressin. Among these neurons are the paired Leydig cells, which have previously been identified to play a neuromodulatory role. Future work will explore the physiological properties and connections of the neurons recognized by antibodies against vasopressin.

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P 106

Mechanisms that initiate and terminate animal locomotion

As in other animals, locomotion in leeches can be initiated and terminated by brief triggers, which may include individual neurons in the brain. The duration of the evoked activity may be prolonged and is variable. In the isolated leech nerve cord, stimulation of a single peripheral nerve usually evokes swim episodes that comprise 10 to 40 cycles of motor neuron bursts. Specific mechanisms that time the duration of the episodes are unknown; however; in the leech there is a positive correlation between the extent of a preparation (number of nerve cord ganglia) and swim duration. Preparations that comprise fewer than six segmental ganglia rarely exhibit fictive swimming, whereas those that include most of the nerve cord, excluding the rostral-most ganglia, generate the longest episodes. We have previously described a conductance-based neuronal model of the leech swim circuits (www.neurodynamix.net) that includes all of the major experimentally-defined elements, including a trigger neuron, gating neurons, the central oscillator circuit and positive intersegmental feedback between excitatory interneurons. This model simulated major physiological findings, including cycle period, intersegmental phase lags, and swim initiation and termination by brief activation of a single trigger neuron. We now report that this model replicates two additional experimental findings: 1) Swim duration is positively correlated with the number of active ganglia, with swim initiation failing when there are fewer than three ganglia. And, 2) swimming can be evoked within 2 s after swim termination. The duration of the second swim episode is correlated with the interval between termination and reinitiation. These modeling results support our view that swim maintenance in leeches arises, in part, from reciprocal excitation between a set of intersegmentally distributed interneurons whose activity is limited by impulse adaptation and synaptic fatigue.

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P 107

Iterated Connections Reveal Functional Segmentation in C. elegans Locomotor Network

Seventy five motorneurons of eight classes innervate the body musculature that propels Caenorhabditis elegans with dorsoventral undulations. These motorneurons receive input mostly from five pairs of interneurons and are synaptically interconnected to create a “motorneuronal network”. To date, only the anterior half of the motorneuronal network of one animal, spanning 42 motorneurons, has been reconstructed from TEM micrographs . This reconstruction was recently reexamined to provide a connectivity data set that is unavailable for any other animal model. We have analyzed this data set to identify iterating patterns of connectivity based on the position of each motorneuron with respect to the muscle fibers it innervates (perimotor position). We therefore remapped the position of every motorneuron based on the muscles it innervates along the body. All the connections made by each motorneuron were than expressed according to their relative position to other motorneurons of the same class. We filtered out connections that occurred only once in the data set and described a typical connectivity pattern for each class. Using cluster analysis, we also found that VA, VB and VD classes exhibited two distinct sub-classes with slight differences in connectivity. We found that connections in the data set are significantly more iterated compared to 500 computer-generated networks in which the targets of connections were shuffled. In fact, most (74 – 91%) of the connections made by each motorneuron iterate within its class or sub-class. This enabled us to describe a single repeating segment that contains 11 motorneurons. Iterating this segment six times along the body of a nematode gives a segmented model of the motorneuronal network. We are using this model network to give context to activity recorded in motorneurons during locomotion, to design experiments and to simulate network activity.

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Leg Muscle Activity and Timing in the Forward and Backward Walking Stick Insect Carausius morosus

Walking kinematics of stick insects differ between forward and backward walking. A first step towards understanding the neuronal basis for kinematic changes such as these observed in adaptive behaviors, and gaining insight into whether specific sensory influences on motor activity known from reduced preparations (Büschges et al. 2008; Gruhn & Büschges, 2008) also serve in the generation of stepping movements in vivo, is to describe leg muscle activity and timing in these behaviors. We report phasing of EMG activities and latencies of the first spikes of three pairs of stick insect middle leg antagonistic muscles (protractor/retractor coxae, levator/depressor trochanteris, extensor/flexor tibiae) relative to the precise electrical touch down and lift off signals of the tarsus. Forward walking stance muscle activities (depressor, flexor and retractor) were tightly coupled to touch down, beginning on average 95ms prior to, and 15 and 27ms, resp., after touch down. Forward walking swing muscle activities (levator, extensor and protractor) were less tightly coupled to lift off, beginning on average 113, 72, and 32ms, resp before lift off. In backward walking only the protractor/retractor muscles reversed phasing compared to forward walking, with the retractor being active during swing and the protractor during stance, while activities and spike latencies of the other muscles remained the same. Comparison of intact animals and reduced preparations during forward straight walking showed only small alterations in EMG activity and first spike latencies. Changing body height altered intensity, but not timing of depressor muscle activity, most likely due to changes in leg joint loading. Finally, except for the short latency of flexor activation at the transition from swing to stance, timing of motor activity during stepping is in accordance with previous results on the influence of movement and load feedback from reduced preparations. Supp. by DFG grants Bu857/8,10,11.

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P 109

Reinforcement of Movement in Local Joint Control Depends on Intersegmental Signals and the Task to be Generated

The motor output for walking that drives individual legs during walking arises from the interaction of descending signals from the brain and intersegmental signals with local sensorimotor networks of the segmental leg muscle control system. It is not clear, how the motor output for adaptive motor behaviors, like forward (fw) or backward (bw) walking, or turning, is produced. We addressed this issue for sensorimotor processing in the femur-tibia (FT-) joint control network of the stick insect Carausius morosus by stimulation of the femoral chordotonal organ (fCO) in a front (fl), middle (ml) or hind (hl) leg, while recording the activity of corresponding tibial motoneurons (mn) and muscles in animals that were walking on a slippery surface with all or a subset of their remaining legs (Gruhn et al. 2006). Turning was induced by optomotor stimulation and fw or bw walking by tactile stimulation of the abdomen or antennae, respectively. We found that reinforcement of flexor activity induced upon fCO flexion signals, the so-called active reaction (AR; Bässler, 1988), was generated depending on the activity of neighboring legs in a segment and task dependent manner. In fw walking ml, ARs were generated in 62% (N=6) with ipsilateral fl and the contralateral legs present and stepping, and in 58% (N=5) when only the ipsilateral fl was present and stepping. Its probability was reduced to 2% (N=3), when only contralateral legs were present and stepping. In fl of animals with five walking legs, ARs were generated during fw (N=7;68%) but not during bw walking (N=7;11%), in ml ARs were generated for both walking directions (N=7; fw49% and bw37%), while in hl neither in fw nor in bw walking (N=8,<20%). During turning ARs were generated in inside turning (N=9;58%), but not in outside turning ml (N=8;8%). Our results indicate that intersegmental signals from stepping legs and task-specific modifications in local sensori-motor processing play a prime role in generating adaptive locomotor behavior. Supp. by DFG grant Bu857/10.

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Working memory in the stick insect Cuniculina impigra

Walking animals that do not find a foothold perform searching movements (SMs) with the respective leg. SMs are rather stereotypic, cyclic movements. Here we explore changes in space and time in the SM pattern upon a one-time disturbance by an obstacle. Experiments were performed on stick insects (Cuniculina impigra) with a single intact foreleg that was restrained to move in the vertical plane. A stick was moved into the leg's path of movement such that it was touched one time by the distal tibia. After touching the leg continued with SMs. Muscle activity was monitored by EMG recordings from the levator and depressor trochanteris. Upon disturbance, animals shifted their SMs towards the position where the disturbance had occurred and the amplitude of SMs decreased significantly. These changes were independent of where in the leg's trajectory the stick was touched, e.g. in its upper or lower portion. Animals performed altered SMs for several seconds, during which the movements slowly shifted back to the original position with increasing amplitudes .The ratio between integrals of levator and depressor trochanteris muscle activity changed upon disturbance and was related to the direction of shift of SMs. Duty cycles and phase relationships of levator and depressor trochanteris did not change clearly. Cycle length decreased upon disturbance and gradually increased again. The directional motor response that outlasts the short stimulus for some time might indicate some sort of short term working memory that provides information about the disturbance as such and its position.

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P 111

Anti-β-tubulin immunohistochemistry on thick transverse ganglion sections after retrograde labeling of mesothoracic leg nerves of the stick insect Carausius morosus

During walking stick insects legs are moved by contractions of antagonistic muscles. For the understanding of how the nervous system controls walking it is essential to identify all motoneurons and modulatory neurons that innervate leg muscles and characterize their morphological and physiological properties. Here we attempt to describe the morphology and the anatomical integration of these neurons into the respective ganglia because morphology determines connectivity and affects the integration of inputs to a neuron. Neuroanatomical studies based on cobalt backfills of nerves that contain axons of leg motoneurons in stick insects already revealed that some leg muscles are innervated by surprisingly many motoneurons [1]. However, these studies suffer from a lack of detail. Especially, they do not provide information about the arborization pattern of neurons in relation to identified neuropil structures, in which, e. g., sensory afferents are known to terminate. To find a remedy, we combined fluorescent dye backfills of nerves with anti-tubulin immunohistochemistry in combination with high-resolution optical imaging. In the mesothoracic ganglion of the stick insect lateral nerves or branches of the main leg nerve were backfilled with fluorescence dextran dyes. Transverse vibratom sections of ganglia were labeled with monoclonal anti-β-tubulin antibodies (mouse, T4026, Sigma) to visualized landmarks as, for example, prominent longitudinal fiber tracts and neuropil regions. Our study reveales that almost all leg muscles are innervated by a higher number of motoneurons than previously published. We show motoneuron cell body positions and clusters and differences in depth and pathways of primary neurites and finer arborizations within the neuropil. Besides, we show the structure and terminal regions of sensory afferents as, for example, hair plates that have axons in the examined leg nerves. References: [1] Storrer et al. (1986) Zool. Jb. Physiol. 90:359-374

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SyntheticAnt, an autonomous robotic forager that models ant behaviour

Autonomous navigation, and in particular of unexplored territories, remains an open challenge in the field of robotics. Moreover, it is not yet well understood what the biological mechanisms capable of generating allothetic behavior from local information are. To date, many neurobiologically based navigation models for mobile robots have been proposed, but only few of them have been implemented in real robots. Insect navigation can provide a simple guideline for systems able to perform sophisticated navigation-related tasks, such as path integration, landmark navigation or foraging, but using few computational resources. Here we study different aspects of insect navigation by building SyntheticAnt. SyntheticAnt is capable of visually and chemically sense a source of food, use navigational strategies that lead it to the food location and a method to find its way back to the nest. We have developed a neuronally based model of a foraging ant and have implemented it in the opensource neural simulator iqr. By combining the information provided by a chemosensor array and a wind vane, SyntheticAnt can detect the incoming direction of an odor plume originated by a feeder inside a wind tunnel. SyntheticAnt is capable of navigating the environment by mimicking moth chemotaxis. Simultaneously, SyntheticAnt uses an onboard camera to sample the environment and identify visual cues while a memory system stores their features together with proprioceptive information, making SyntheticAnt capable of homing using a straight path once it reaches the feeder. Additionally, information gathered previously allows SyntheticAnt to locate the feeder in the absence of wind, and to find its way home if placed at arbitrary locations. Experimental results show that using the combination of chemical sensing, visual navigation, proprioception and path integration, SyntheticAnt is able to effectively close the foraging loop and account for ant foraging behavior.

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P 113

Reproducing basic patterns of insect locomotion and transitions between them by means of a neuronal network model

Locomotion in insects requires coordinated working of the segmental neuronal networks that control the limb movements. Recent experiments have shown that sensory signals originating in the extremities play a pivotal role in controlling locomotion patterns. Based on these results, we constructed an inter-segmental model comprising the pro-, meso-, and meta-thoracic local networks, their interconnections and the main sensory inputs. In the model, the local networks are uniform, and each of them consists of a central pattern generator (CPG) providing the rhythmic oscillation for the protractor-retractor motor systems, the corresponding motoneurons (MNs), and inhibitory local interneurons between the CPGs and the MNs. Between segments, the CPGs are connected cyclically by both excitatory and inhibitory synapses that are modulated by the aforementioned sensory inputs. It was assumed that each of the CPGs oscillated spontaneously with the same centrally imposed period. Implementing this structure, and the properties of the CPGs and other neurones, as well as synapses as a computer model, we found in the simulations that the model was capable of reproducing basic patterns of locomotion such as those occurring during tripod (3pod) and tetrapod (4pod) gaits. The activity patterns of the pro- and retractor MNs appropriately reflected their respective behaviour. Further investigations with the model revealed a number of elementary neuronal processes (e.g. synaptic inhibition, or changing the synaptic drive at specific neurones) that were necessary, and in their entirety sufficient, to bring about a transition from one type of gait to the other. The majority of these processes is of sensory origin, even though some changes are also required in the centrally controlled synaptic activities. The main finding of this study is that exactly the same mechanism underlies the transition between the two types of gait irrespective of the direction of the change.

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A Dynamical Systems Approach to Behavior Modulation and Decision Making

Despite many technical advances, current mobile robots lack true autonomy and are restricted to constrained environments and tasks. It is often convenient to segment a complex robotic control task into component behaviors that are easy to develop and test in isolation. In practice, a full repertoire of behaviors, each triggered by some combination of sensory input, can be challenging to tune and sequence under real-world conditions. Animals rely on the multi-modal sensory integration and decision-making capabilities of their brains to reliably trigger, modulate, and provide context to their low-level, reflexive motor control systems. They do this in dynamic and uncertain environments that challenge the sensing and autonomous capabilities of existing robots. We are investigating a class of nonlinear dynamical systems called stable heteroclinic channels (SHCs) as a biophysically-plausible solution to how a brain-like system might smoothly and reliably sequence and switch between behaviors in the presence of large amounts of time-varying noise and sensory input. An SHC is composed of saddle nodes interconnected in phase space by their unstable manifolds to create a globally-attracting multi-dimensional stable manifold. Each state of an SHC can be thought of as the average activity of a neural population that excites or inhibits the others. The connectivity of the network defines an ordered pattern of activity flowing from one population subset to the next. Sensory input serves to modulate the speed and timing of the unidirectional state transitions. We implement decision making via by clusters of mutually-inhibitory populations that integrate their respective time-varying sensory evidence while competing in a winner-take-all manner. Unlike many other types of neural networks, SHC networks are easier to characterize and we show that they can be designed in a straightforward manner. We also describe how multiple SHCs can be coupled together or extended hierarchically.

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P 115

Crayfish select escape strategies based on external conditions and internal states

The behavioral and neural responses of juvenile crayfish to visual danger stimuli can be measured non-invasively using a combination of standard videography with recordings of electrical field potentials. When juvenile crayfish approach a food odor release point in an aquatic tank, they respond to sudden threat signals (shadows) with discrete behavioral outputs: freezing or tail-flipping. Tail-flips are mediated by activation of the medial giant interneurons and propel the animals away from the food source. Crayfish assess different behavioral options and make decisions based on the features of the threat stimulus, the value of the expected resource, and their current internal state. If the value of the expected food reward is high, tail-flipping is suppressed in favor of freezing. High resource value is less effective in suppressing tail-flips, however, when paired with a strong predator signal. Freezing also dominates in hungry animals whereas satiated animals produce mostly tail-flips. In addition, crayfish taken from communal tanks, where competition over food is high, freeze more and tail-flip less than isolated animals. This suggests that nutritional states and recent social experiences affect decision-making neural circuits; intrinsic needs and reward value are balanced against predation risk to produce the most desirable behavioral choice. Since tail-flipping is controlled by accessible neural circuitry, we have now begun to investigate the neural processes underlying value-based escape behavior in crayfish. Supported by National Science Foundation grant IOS-0919845 (JH).

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P 116

Neural and mechanical mechanisms of jumping in planthopper insects

Planthoppers (Issus coleoptratus, Hemiptera, Issidae) are some of the most accomplished jumpers of all insects. High speed imaging at 7500 frames per second shows that they accelerate in 0.8 ms to a take-off velocity of 5.5 m s-1. A jump displaces them a horizontal distance of 1.1 m while they experience a force of 719 g. A jump is produced by a catapult mechanism powered by huge trochanteral depressor muscles in the thorax that control the coxo-trochanteral joints of the two hind legs. The short hind legs represent only 65% of the body length and move in the same plane beneath the body to propel a jump. The motor pattern for jumping consists of three phases. First, the two hind legs are moved into their cocked position by levation of the coxo-trochanteral joints. Second, a sequence of motor spikes lasting many seconds, begins in the power producing muscles. The spikes are tightly synchronised in the two pairs of motor neurons supplying the left and right sides. A small part of the depressor muscle is innervated by an unusual neuron with a large cell body, a large, blind-ending dendrite, and a giant axon 50 µm in diameter, the largest found in any insect. During these prolonged contractions the hind legs remain stationary, but paired bow-like parts of the thoracic skeleton are progressively bent. These structures, which are composites of hard cuticle and the elastic protein resilin, store the energy produced by the slow contractions of the depressor muscles. Finally, the stored energy is suddenly released and powers the rapid movements of both hind legs which are synchronised to within 30 µs of each other by a mechanical coupling. This linkage ensures that when one hind leg moves the other will also move at the same time, and only comes into play when both are cocked into their initial jumping position. These neural and mechanical mechanisms allow the muscles to generate movements that are both powerful and rapid, thus enabling the insect to be launched at high velocity.

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Unravelling the Central Pattern Generator for Cricket Singing

Cricket species use a variety of specific song pattern for intra-species communication. Males produce sound pulses with movements of their forewings which may be organised into complex species-specific patterns of chirps and trills. Although singing is a key feature in crickets, very little is known about the neuronal generation of the song patterns. Understanding the central pattern generator (CPG) for singing at the level of identified neurons will not only provide insight into the control mechanisms of one of the most conspicuous insect behaviours. Linked with a genetic approach this may also reveal which neural changes caused song pattern modification during evolutionary segregation of cricket species. Currently we identify the components of the singing CPG in Gryllus bimaculatus by intracellular recording and staining of interneurons in the CNS of fictively singing males. Singing is elicited by injection of Eserine into the brain and the singing motor pattern is monitored by extracellular motoneuron recording. So far we identified intersegmental descending interneurons in the metathoracic and ascending neurons in the abdominal ganglia as elements of the singing CPG. These interneurons discharged in phase with the singing rhythm and perturbing their activity with intracellular current injection modified or reset the motor pattern. (Supported by the BBSRC)

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Pattern generation and coordination in cockroach locomotion

The cockroach is renowned for its remarkably stable, yet rapidly adaptable, locomotion. This has been crucial for its evolutionary success. It is also a major inspiration for the development of mathematical models of multi-legged locomotion, as well as biologically-inspired robotics. Two extreme schemes have been proposed for locomotion control; in the first, central pattern generator(s) (CPGs) coordinate rhythmic limb motion. In the second, motor activity depends on sensory inputs (feedback loops). Here we utilize a combined experimental and theoretical approach to investigate the relative importance of inter-segmental afferents versus CPG interconnections for the coordination of cockroach locomotion. We simultaneously recorded the coxae levator and depressor motoneurons in the thoracic ganglia of a walking cockroach, while sensory feedback was completely blocked, or allowed only from one specific intact stepping leg. We observed a tripod-like activity gait in the absence of sensory feedback, suggesting the existence of central feed-forward control. In addition, we show transient stabilization of phase differences between the middle and hind thoracic motoneurons following individual steps of a front leg, suggesting that phasic information from a stepping leg can stabilize phase relations between the other components of the circuit activating the different legs. Data were further analyzed using stochastic models of coupled oscillators and maximum likelihood techniques to estimate underlying physiological parameters, such as uncoupled endogenous frequencies and the strength and direction of coupling. Our findings indicate that descending ipsilateral coupling is stronger than ascending, while left-right coupling in both the meso- and meta-thoracic ganglia appears to be symmetrical. A comparison with recent findings in stick-insects may indicate different inter-segmental coordination strategies in the two insects that exemplify opposite extremes of a fast-slow locomotion continuum, while sharing much of the neural and body architecture.

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403 **P 119**

Co-activation of the central pattern generators for flight and walking

Locust walking and flying are generated by central pattern generators (CPGs) that are adjusted to the motor pattern of intact animals by phasic sensory inputs. In freely behaving locusts, the two motor patterns are mutually exclusive. However, since leg movements during flight may be coupled to the wing-beat cycle we analyzed the extent to which the CPGs for walking and flight are interconnected. We bath applied the muscarinic agonist pilocarpine, and the biogenic amines tyramine respectively octopamine to the CNS of deafferented locusts while monitoring motor units in cut nerves controlling the swing and stance phase of the hind leg together with motor units that activate hind-wing elevator and depressor muscles. Fictive walking is evoked by low concentrations (10-4 M) of the muscarinic agonist pilocarpine, whereas higher concentrations are required to elicit fictive flight (10-3 M). Flight did not suppress walking motor activity and the two patterns were produced simultaneously (18 out 21 preparations). Frequently, at least one leg motoneurone was temporally coupled to the flight rhythm. Similarly, bath application of the biogenic amine tyramine also induced both fictive walking (at 10-3 M) and flight (at 10-2/ 10-1 M) with rare temporal coupling of units (5 out of 12 preparations). Contrasting this, the neuromodulator octopamine readily evoked flight (10-2 - 10-1 M) but mostly failed to elicit walking (80%, 12 out of 15 preparations). Leg motor units were temporarily coupled to the flight rhythm throughout the whole flight sequence. Flight motor activity elicited by natural stimuli (wind) served as a reference. In 9 out of a total of 14 preparations coupling of walking motor units to the wing beat cycle was observed. Our results indicate that the degree of coupling between the walking and flight CPGs is subject to aminergic modulation.

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The Origin of behavioral bursts in decision-making circuity

The timing of many animal behaviors are characterized by bursts of activity separated by long periods of inactivity. Bursty dynamics is widespread (1-6), but only recently has there been a modeling effort to understand its origin from a behavioral point of view (7,8). In the proposed model, behavioral bursts are the consequence of an internal decision-making process, where tasks are executed according to a queued priority list. In this study we have experimentally examined this link in Drosophila melanogaster, by using targeted mutations of structures known to disrupt decision-making (9). To characterize burstiness, we found that the distribution of intervals between activity bouts of wild-type Drosophila are well described by the Weibull distribution, a common distribution of bursty dynamics in complex systems. The Weibull shape (k) parameter can thus be used to quantify and compare burstiness between different genotypes and/or conditions. In addition, we show that the bursty dynamics is mainly determined by the inter-activity distribution and not by memory effects, similarly to human dynamics (5). To examine the connection between decision-making circuit and behavioral burstiness, we altered dopaminergic signaling or selectively silenced parts of the mushroom body (9), and found that the flies' inherent burstiness changes, a result consistent with the proposed model. 1. Wiens et al., Ecology. 76:663(1995) 2. Cole, Animal Behaviour. 50:1317-1324(1995) 3. Lo et al., P Natl Acad Sci USA. 101:17545-8(2004) 4. Edwards et al., Nature. 449:1044-8(2007) 5. Goh & Barabási, EPL. 81:48002(2008) 6. Sims et al., Nature. 451:1098-102(2008) 7. Barabási, Nature. 435:207-11(2005) 8. Vázquez et al., Phys Rev E 73:036127(2006) 9. Zhang et al., Science. 316:1901-4(2007)

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Identification of neurons that affect feeding behavior in adult Drosophila melanogaster

Feeding is an essential but complicated behavior during which an animal must process sensory cues to identify nutritious food and avoid toxins. Feeding is also dependent on additional factors such as the internal state of the animal, i.e., how energy depleted or hungry the animal is. Finally, ingesting food requires activating the right motor program. Identification of the neural circuits regulating feeding behavior in Drosophila may reveal general principles about how external cues and internal state are integrated to modify a motor program. To identify new components of the feeding circuit, I place starved flies on food loaded with blue dye. They readily eat this food, while fed flies do not. For the screen, I activate small groups of genetically targeted neurons in fed flies and select for animals that now eat as if hungry. I have screened >1500 GAL4 lines (G. Rubin lab) with the neural activator UAS-dTrpA1 (a temperature-sensitive cation channel) and looked for fed flies that now eat blue food. 9% of lines show increased feeding. Some of these lines have highly penetrant phenotypes with mild increases in feeding, while others have weaker penetrance but a more extreme phenotype. Further analyses have identified subclasses of hits based on anatomy and additional behaviors such as feeding of non-nutritive substances, which may indicate a thirsty rather than hungry fly. The GAL4 lines that cause overeating with activation were also crossed to the inhibitor UAS-Shibire ts1 to block transmission and behavior of starved flies was assessed. I see reciprocal phenotypes in some of the lines, which may suggest that these neurons are critical for the decision to feed. With the behavioral screen I have developed, I hope to isolate potential command interneurons rather than sensory or motor components. With a combination of genetics, behavioral analysis, and electrophysiology, I will further characterize how these neurons reflect the internal hunger state of the animal.

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676 **P 122**

Neurons affecting innate grooming behavior in Drosophila

We sought to identify neural circuitry driving fruit fly grooming to gain insight into how nervous systems generate complex motor behaviors. Mechanosensation of dirt on specific body parts induces stereotyped cleaning movements characterized by site directed leg sweeps. In contrast, we found evidence of higher order structure to grooming as placement of dust on the entire body revealed a prioritization of head cleaning before that of the rest of the body. We combined a high throughput-cleaning assay with Drosophila tools for disrupting neurotransmission in genetically selected subsets of neurons and identified circuitry driving grooming behavior. Specifically, we screened a library of 4,171 GAL4 lines (Pfeiffer 2008) that drove expression of the acute neural inhibitor UAS-Shibirets1 in different brain and thoracic expression patterns to identify those that could perturb the cleaning response to dust. The screen revealed categories of lines that failed to remove dust from discrete body parts and groups that lacked the coordinated progression of grooming behavior. Anatomical and behavioral analysis revealed that we isolated neurons in sensory systems, interneurons, and motor outputs. Interestingly, secondary analysis of the grooming defective GAL4 lines using neural activators uncovered neurons that are sufficient to drive discrete modules of grooming behavior in the absence of mechanosensory stimuli. We are now piecing this circuitry together by combining behavioral analysis, anatomical approaches, and calcium imaging to generate a model for how grooming behavior is structured in the nervous system. Consistent with our data and the work of others we postulate that grooming circuitry consists of local networks of neurons that act autonomously to drive specific components of the behavior and interneurons connecting these local modules to coordinate a cleaning response. I will present data on the stereotyped structure of wild type grooming behavior, screen results, categorization of grooming defective lines, and identification of grooming-specific neurons.

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Role of potassium currents in the recruitment of drosophila motoneurons

Identified drosophila larval motoneurons display distinct functional properties, including differential expression of potassium channels, that affect recruitment during behavior. Although the drosophila larval motor system has provided a powerful genetic model for exploration of synapse development and function, relatively little information is available about the properties of the motor neurons that contribute to activation during behavior. We used in situ whole cell recordings to describe basic firing properties and voltage-gated currents of the larval motor neurons and to monitor motor neuron activity during fictive crawling. Potassium currents, in particular, have a marked influence on recruitment and firing properties. Larval motor neurons express both calcium-dependent and calcium-independent potassium currents. Ether-ago-go (EAG) contributes to transient and sustained currents of both types, as demonstrated by analysis of mutant and EAG-RNAi expressing neurons. Shal contributes significantly to the transient (A-type) calcium-independent current. A comparison of type 1b motor neurons (large synaptic boutons on individual muscles) and type 1s motor neurons (small synaptic boutons on several muscles) reveals a higher threshold and significant delay to the first spike in response to current injection for the latter. Shal current plays a major role in the delay to spike, as demonstrated by selective expression of shal-RNAi. Differences between the two larval motor neuron types are relevant to their recruitment during fictive crawling. Simultaneous whole-cell recordings reveal similar synaptic drive for type 1b and 1s motor neurons innervating the same body wall region, but higher threshold and longer delay to spike for the latter.

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Schaefer, Jennifer

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Srinivasan, Subhashini

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P 124

Functional Importance of Synaptic Modulation in a Central Pattern Generator Network

Neuromodulators modify the behavioral output of neural networks by altering the firing properties of multiple network neurons and synaptic strengths at multiple network sites. We are examining the significance of amine modulation of synaptic strength in the pyloric network of the stomatogastric ganglion. The pyloric LP neuron makes synapses onto 3 groups of follower neurons: it inhibits the PY and VD motor neurons and provides the sole inhibitory feedback to the pyloric pacemaker kernel AB/PD group to limit cycle frequency. Here we study the functional consequences of modulatory changes in LP synaptic output on PY and VD firing phase and the pyloric cycle frequency. Using realistic waveforms to drive the pre-synaptic LP neuron, we have shown that dopamine (DA) enhances LP→PY inhibition, but this has no effect on PY firing phase. DA and Oct strengthen while 5HT weakens LP→ PD inhibition. Paradoxically, the DA-strengthened LP→PD synapse loses its ability to regulate the pyloric cycle period, while the 5HT-weakened LP→PD synapse has a stronger influence on cycle period. These effects arise from monoamine modulation of the intrinsic firing properties of neurons and the strength of other synapses that modify the offset phase of LP firing. DA phase-advances LP activity into the refractory period of the AB/PD oscillations, where the increased LP inhibition has no further effect. 5HT phase-delays LP activity to a very sensitive period of AB/PD oscillations, where even the weakened LP synapse significantly slows the pacemaker frequency. Finally, DA and Oct strengthen while 5HT weakens the LP→VD synapse, but again these synaptic changes do not significantly alter the phasing of VD activity in the pyloric rhythm. VD firing after pacemaker inhibition is controlled by monoamine modulation of its intrinsic firing properties. By examining modulation of all synaptic output of a single important network neuron, we demonstrate that the functional consequences of specific modulatory changes in synaptic strength depend on the multiple, integrated network actions of the modulator on neuronal excitability and on other network synapses. Supported by NIH NS17323.

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Harris-Warrick, Ronald

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P 125

Pacemaker neuron and network oscillations depend on a neuromodulator-regulated linear current

Linear leak currents have been implicated in the regulation of neuronal excitability, generation of neuronal and network oscillations, and network state transitions. Yet, few studies have directly tested the dependence of network oscillations on leak currents or explored the role of leak currents on network activity. In the oscillatory pyloric network of decapod crustaceans neuromodulatory inputs are necessary for pacemaker activity. A large subset of neuromodulators is known to activate a single voltage-gated inward current IMI, which has been shown to regulate the rhythmic activity of the network and its pacemaker neurons. Using the dynamic clamp technique, we show that the crucial component of IMI for the generation of oscillatory activity is only a close-to-linear portion of the current-voltage relationship. The nature of this conductance is such that the presence or the absence of neuromodulators effectively regulates the amount of leak current and the input resistance in the pacemaker neurons. When deprived of neuromodulatory inputs, pyloric oscillations are disrupted; yet, a linear reduction of the total conductance in a single neuron within the pacemaker group recovers not only the pacemaker activity in that neuron, but also leads to a recovery of oscillations in the entire pyloric network. The recovered activity produces proper frequency and phasing that is similar to that induced by neuromodulators. These results show that the passive properties of pacemaker neurons can significantly affect their capacity to generate and regulate the oscillatory activity of an entire network, and that this feature is exploited by neuromodulatory inputs.

Nadim, Farzan

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Biologically realistic limb coordination during walking in the absence of central connections between legs

Neuronal control of interlimb coordination during walking is not fully understood. Load and position cues can influence stepping coordination between legs, but it is unclear if central connections between legs are required. To test this, we created a crayfish model in the simulation environment AnimatLab. In the model, all legs are identical, and receive sensory feedback from three sources: position sensors at each joint, a contact sensor at the dactyl, and a load sensor. All legs are driven by individual oscillators. That is, all legs function independently of each other with no direct connections between the legs. The model can produce realistic walking behavior without load feedback. The only sensory cue that is necessary is from the contact sensors. In walking simulations, legs nearest the center of mass showed a preferred coordination with ipsilateral and contralateral neighbors. Legs farther away from the center of mass displayed more variability in coordination. Contact information was also sufficient to change the characteristics of leg oscillations, i.e. cycle periods and stance durations. We also tested the effects of limb amputations. In intact models, coordination between adjacent ipsilateral legs is predominantly alternating. After amputation of a leg, the two intact neighboring legs assume an alternating coordination. These results are very similar to those observed in actual crayfish. These findings suggest that interlimb coordination in multi-legged animals does not have to be integrated into the neuronal network that produces walking. Instead, coordination can be an indirect result of the coupling of individual leg oscillators through the environment.

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P 127

Homologous interneurons have distinct functions in the generation of similar rhythmic motor patterns

The nudibranch molluscs *Melibe leonina* and *Dendronotus iris* exhibit similar swimming behaviors consisting of rhythmic lateral body flexions. The swim central pattern generator (CPG) in *Melibe* consists of 2 bilaterally symmetric swim interneurons (Si1 and Si2) that fire bursts in alternation with their contralateral counterparts because of mutual inhibition (Thompson & Watson, JEB, 2005). Here we found that a distinct neural mechanism produces a similar motor pattern in *Dendronotus*.

In *Dendronotus*, we identified putative Si1 and Si2 homologues. The putative Si2 homologues in the pedal ganglia were mutually inhibitory and fired in an alternating bursting pattern. In contrast, putative Si1 homologues in *Dendronotus* were not mutually inhibitory and were not rhythmically active during the motor pattern. We named them the Cerebral Ipsilateral Pedal (CIP-1) neurons because of their location and characteristic axon projection to the ipsilateral pedal ganglion.

Unlike the *Melibe* Si1, the *Dendronotus* CIP-1 bilaterally excited other swim interneurons. Depolarization of CIP-1 initiated a swim motor pattern or accelerated an ongoing motor pattern. Hyperpolarization of CIP-1 slowed the rhythm. Thus, the *Dendronotus* CIP-1 is not a member of the swim CPG, but plays a regulatory role in rhythmic motor pattern generation.

Si1 and CIP-1 differed in their electrical connectivity. In *Melibe*, synergistic bursting of Si1 and Si2 on the same side was caused by ipsilateral electrical coupling that was approximately 10 times stronger than the coupling between the contralateral counterparts. In contrast, in *Dendronotus*, CIP-1 appeared to have evenly-distributed electrical coupling with swim interneurons on both sides. Thus, although the rhythmic behaviors of *Melibe* and *Dendronotus* appear similar and the nervous systems contain homologous neurons, there are important differences in the organization of the CPGs that produce the motor patterns.

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Newcomb, James M.

New England College

Katz, Paul S.

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P 128

Animal-to-animal synaptic variability within a central pattern generator affects responses to neural injury.

Modeling studies suggest that similar activity patterns can be produced by circuits having different sets of synaptic strengths (Prinz et al, Nat Neurosci 7, 2004), but the behavioral significance of such differences has not been explored. We report here that synaptic strengths within a central pattern generator (CPG) can differ for individual animals exhibiting similar behavior, but that different responses are revealed when the neural circuit is challenged with a lesion. We previously showed that the swimming behavior in the mollusc (i)Tritonia diomedea(i/) was impaired by lesion of the pedal-pedal commissure and spontaneously recovered after 15 hours (Sakurai and Katz, J Neurosci 29, 2009). Here we found that individuals differed in their susceptibility to lesion and extent of recovery. Furthermore, those differences correlated with the strength of particular synapses within the swim CPG. Upon lesion, there was a reduction in the number of flexion cycles in the swim both (i)in vivo(i/) and (i)in vitro(i/); the extent of reduction differed among individuals. Such individual variability was inversely correlated with synaptic strength between two CPG neurons (C2 and VSI). After lesion, individual variability was seen in the extent of recovery of the swim motor pattern. The variability in recovery was correlated with the amplitude of the excitatory synaptic responses in VSI when C2 was stimulated together with another member of the swim CPG, the serotonergic DSI. This suggests that neuromodulatory actions may be involved in recovery. These results indicate that although individual differences in the CPG synapses are not correlated with differences in the normal behavior, they do underlie differences in responses when challenged with a lesion. Thus, while animal-to-animal variability may not have an apparent effect on normal operation of a neural circuit, it may be exposed if that circuit is pushed into another functional state, such as that caused by a lesion.

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P 129

The environmental skeleton: Tension-based locomotion in a soft-bodied climbing insect.

An outstanding problem in animal locomotion is to explain how soft animals control their movements. Most models of soft bodied locomotion assume that force transmission is mediated by a hydrostatic skeleton. Using a variety of experimental approaches on Manduca sexta we now show that caterpillars do not necessarily rely on hydrostatics for crawling and climbing. We have identified five novel results relating soft body mechanics to the neural control: (1) Manduca caterpillars crawl horizontally and climb vertically using the same kinematic gait. (2) In electromyographic recordings from identified muscles in freely crawling Manduca the activity of major dorsal and ventral muscles are not functionally antagonistic and there is substantial overlap in activity between longitudinal and oblique muscles in different segments. (3) The first measurements of caterpillar surface reaction forces at multiple contact points suggest a new model of caterpillar crawling in which compressive forces are carried by the substrate; locomotion is based on the sequential release of tension through controlled changes in grip. (4) Using synchrotron-sourced phase contrast X-rays, we found that the gut slides forward in mid-body segments before the body wall itself, thereby causing a shift in the center of mass of the animal during the stance phase. (5) Manduca body volume is not necessarily constant during movements and pressure is not uniform in the body or well-correlated with movements. Together these findings suggest that, in contrast with the hydrostatic systems employed by annelids and mollusks, Manduca uses an "environmental skeleton" that neither depends on controlling internal pressure nor requires antagonistic muscle activity. One advantage of this strategy is that Manduca can remain comparatively soft and conform to the substrate for stability and camouflage. This discovery is being used in the design, construction and control of a new class of terrestrial, soft material robots.

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Simon, Michael

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Lin, Huai-ti

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611 **P 130**

Bio-inspired postural control for a standing quadruped robot

Postural stability, body balance and orientation are fundamental factors that condition the success of locomotion in complex natural environments, a requirement if one wants to achieve autonomous robotic legged locomotion. A requirement that poses serious control problems, requiring the coordination of a large number of degrees of freedom. Analysis of different species has shown that there are individual central systems for posture and locomotion that interact when required. These kind of posture and balance adaptations are achieved by adapting and correcting the locomotor movements; of innate reflexes ready to be elicited depending on context; and sensory dependent responses that maintain the proper expression of locomotion in specific conditions. In this work we propose a posture control system for a quadruped robot, inspired by the spinal and supraspinal postural networks, integrated with a Central Pattern Generator (CPG) based locomotion system. On this first approach we tackle posture on a quiet standing quadruped quadruped robot, aiming to further generalize the designed system for locomotion. We try to provide our robot with the ability of maintaining balance on inclined and uneven terrains while maintaining the Center of Gravity within the support base; supporting the body for voluntary movements of the trunk and legs; and searching for secure supporting footholds. We design several postural reflexes and responses based on sensory information of different modalities. The robot"s posture is an overall response of somatosensory information. The constituent reflexes and responses work in parallel, competing and coordinating outputs, driving the legs" joints and adapting the robot"s standing posture. The control system is validated through a few experiments, where the robot is subjected to different posture situations ranging from roll and pitch variations to loss of feet support.

Matos, Vitor

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Santos, Cristina

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Model based analysis: A novel approach to understanding behavioral data of rodents

Key to ethological research is the quantification and extraction of higher order characteristics from behavioral data. Here we propose a novel, model driven, approach to the analysis of animal behavioral data, specially focusing on rodents. This approach uses a minimal assumption model of internal states, comprising four homeostatic subsystems: Security, arousal, reward, and cue. These are conceptualized as regulating themselves through the local perception of a gradient map. We estimate the internal states that are driving the behavior based on the trajectories in a controlled experimental setup. Once the corresponding gradients for each subsystem are defined, the actual value (AV) time series can be obtained. We argue that the time series of the AV are a valid and meaningful compression of the behavioral trajectory to a single dimension. We analyze three experimental rodent setups that assess real-world foraging of increasing complexity. Firstly, free exploration of a square arena, secondly, interaction of homing and foraging behavior in an I-Maze, and thirdly, a multiple reward port task with delivery of cues indicating reward in a Y-Maze. Statistical quantification shows that the shape of the arena has a clear influence on the percentage of stays in open space. Our approach supports the interpretation that in an open square arena the rat feels unprotected in the middle and prefers to explore the environment following walls, whereas in the I-Maze and Y-maze this constraint is not present as the space is narrower, leading to a weakening of the relevance of arousal, and an increase of importance of the security gradient. If the task is more dynamic (Y-Maze) security shifts towards places of behavioral relevance. The model driven approach presented here, opens new ways to the quantification, and extraction of meaning from behavioral data, and our results provide a biologically grounded view on how the complex behavioral patterns of rodents can be seen as a results of the interaction of a number of minimal subsystems.

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Orientation & Navigation

468 **P 132**

First evidence of star orientation in insects

Birds and seals are well known to orient to the stars, and we now show that insects also possess this ability. This finding is the result of a series of experiments to determine how straight nocturnal dung beetles roll under the open sky with (1) polarised light from the moon available as the main orientation cue, (2) with only stars available as an orientation cue and (3) with little caps fitted over the dorsal eyes of the beetles to greatly reduce the availability of celestial cues for orientation. The beetles were released with their dung balls from the centre of a circular arena (diameter 3 m) and filmed from above. Rolling tracks were reconstructed from the films and their straightness determined over a radial distance of 120 cm. Tracks of beetles prevented from seeing a full view of the night sky become significantly longer (477 ± 75 cm) than when the beetles are allowed to view either a moonlit (133 ± 2 cm) or a starlit sky (185 ± 12 cm). This indicates that the beetles can orient to the stars as well as to the moon. To test our theory that the beetles are able to use stars for orientation, we studied the orientation performance of beetles in the Johannesburg planetarium. Since the rolling speed of beetles does not change with stimulus condition, the orientation performance of the ball-rolling beetle can be determined by timing the beetles from the moment they leave the centre of the arena until they reach its edge. In the planetarium, the beetles take the same time to reach the edge of the arena, irrespective of whether they roll under the full starry sky (4000 stars and the Milky Way) or with only the Milky Way visible. With only the 18 brightest stars projected on the planetarium dome, the beetles take significantly longer to reach the edge of the arena. This finding represents the first convincing demonstration for the use of the Milky Way for orientation in the animal kingdom, and the first demonstration of star orientation in an insect.

Dacke, Marie (Lund University); Baird, Emily (Lund University); Byrne, Marcus (University of the Witwatersrand)¸Warrant, Eric (Lund University)

Star orientation in a diurnal dung beetle

Nocturnal dung beetles remain the only insects that have been demonstrated to use the polarisation pattern around the moon (Dacke et al. 2003 Nature 424:33) and even the stars (Dacke et al., in prep.) as directional cues for orientation. Supposedly, their highly adapted visual systems – with larger lenses, wider and longer rhabdoms than their diurnal relatives and a tracheal tapetum – enable them to perform this difficult task. We show here, for the first time, that even a diurnal dung beetle without any specialisations for dim-light vision can use the stars to keep a straight course on a moonless night. We compared the orientation performance of the exclusively diurnal Scarabaeus nigroaeneus and the nocturnal S. satyrus when rolling their balls (1) under the open sky on a clear moonless night, and (2) in a control condition with an added artificial light source. For each condition, 20 beetles of each species were individually placed in the centre of a circular arena (diameter 3m). Their outbound tracks were recorded from above and their straightness evaluated by the track length from the centre to the rim of the arena. With the aid of an artificial light all beetles roll in a straight line. While the performance of the nocturnal beetles is equally good under the starry sky, the paths of the diurnal animals are on average 15% longer (i.e. more curved). However, most of the beetles – diurnal and nocturnal – were clearly able to use the stars as an orientation cue. In additional experiments in the laboratory, beetles of both species were tested at a number of different light conditions to determine the minimum light level necessary for orientation. Our preliminary analysis suggests that, once again, the performance difference between the species is smaller than could be expected from the anatomy. We will discuss the implications for our interpretation of visual adaptations to dim light, and of the potential for nocturnal navigation in a large number of insect species.

Smolka, Jochen (Lund Vision Group, Department of Biology, Lund University, Sweden); Baird, Emily (Lund Vision Group, Department of Biology, Lund University, Sweden); Byrne, Marcus (School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, South Africa); Reber, Therese (Lund Vision Group, Department of Biology, Lund University, Sweden); Dacke, Marie (Lund Vision Group, Department of Biology, Lund University, Sweden)

Visually based homing in a nocturnal spider

At the darkest times of night adult males of the Dancing White Lady spider (Leucorchestris arenicola) walk several hundred meters across the Namib Desert sand dunes searching for females. These excursions are round trips starting and ending at a burrow and the spiders thus perform long-distance homing under very dim light conditions. They nevertheless rely heavily on vision. The combined visual fields of the spiders' eyes have a near full panoramic view of the surroundings and only the posterior median eyes are not used for navigation. Calculations of photon catches indicate that the eyes must utilize a combination of spatial and temporal pooling in order to function at night. Using only the basic spatial and temporal resolution of the eyes the spiders would only be able to see the brightest stars in the sky and not any objects in the landscape. Using spatial and temporal summation the spiders would be able to detect larger structures in the landscape. Earlier studies have shown that the spiders use landmarks when homing and indicate that they depend on extended temporal summation that allows them to see such stationary objects in sufficient detail. The exit trajectory of spiders leaving the burrow for the first time within a new area has a conspicuous sinusoidal shape. This behaviour appears analogous to the orientation flights of honey bees and could ensure that the spiders obtain a three dimensional representation of landmarks in the surroundings. If the spiders do more than one excursion during a night, consecutive exits become progressively straighter. This change in trajectory shape indicates that the spiders have the ability to perceive and remember the layout of objects in their surroundings.

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Warrant, Eric

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How desert ants use a visual landmark for guidance along a habitual route

Many animals learn to follow habitual routes between important locations, but how they encode their routes is still largely unknown. Desert ants traveling between their nest and a food-site develop stable visually guided routes that can wind through desert scrub without the use of trail pheromones. Their route memories are sufficiently robust that if a nest-bound ant is caught at the end of its route and replaced somewhere earlier along it, the ant will recapitulate the route from the release site. The panorama appears to allow insects to recognize when they are on a familiar route. A number of mechanisms are known that can provide guidance along a straight segment of a route. But how does an ant encode a curved route along which both the views it sees, and the directions it takes, are constantly changing? The results here suggest that when an ant travels past a landmark on a familiar route, it uses the gradually changing direction of the landmark to trigger a set of associated learnt heading directions. A route through a complex two-dimensional environment could thus be encoded and followed economically if it is divided into panorama-defined segments, with each segment controlled by such a one-dimensional mapping. The solution proposed for the ants would be simple to implement in an autonomous robot.

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P 136

Scanning behaviours in Melophorus bagoti: a window onto the mind of a navigating ant

Foragers of many ant species learn long, visually guided routes between their nest and profitable feeding grounds. The mechanisms underpinning the use of visual landmarks are much studied but less is known about how insects extract visual information from a natural scene. Recently we observed that when the Australian desert ant M. bagoti is disoriented or in an unfamiliar scene, it scans the world by pausing and then rotating on the spot. To further investigate this scanning behaviour and its role in visually-guided navigation, we trained ants to a feeder where the only exit was along a narrow channel. Using a high-speed (300 fps) camera we could record the portion of ants' homeward paths immediately at the channel exit when the natural panaoramic scene was first available to the ant to control their homeward path. Moreover, we could provoke scanning, in the field of view of the camera, by adding large screens which significantly altered the panoramic view of the world to which the ants had become familiar. We see that the number of scans is proportional to the degree of change in the visual scene and our detailed analysis of the scanning behaviour shows it to be saccadic in nature. Further analysis will allow us to correlate the directions adopted during the scan with properties of the visual scene.

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Cheng, Ken

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P 137

Finding home: Inbound searching behaviour in the Australian desert ant Melophorus bagoti

Australian desert ants Melophorus bagoti return home after foraging by means of path integration and visual navigation. If these mechanisms do not deliver them exactly at the inconspicuous nest entrance, they engage in a systematic search. Here we describe the structure of this search pattern in detail. Trained ants ran home from a feeder in the natural visual setting where the navigational error was minimal, and they were captured for tests on a distant test field after they had almost reached their nest. The search pattern consists of loops and is centred on the position where the nest is most likely located. At first, it covers a rather small area, but then gradually extends outwards to cover a larger area. The search density is also adapted to the preceding outbound foraging distance, with longer distances leading to flatter, wider search distributions. Since the visual surround at the time of capture was similar for ants with all outbound distances, we suggest this is an adaptation to the cumulative error of the homing vector. The frequency distribution of segment lengths in the paths of searching ants does not show characteristics of a Lévy walk strategy. Instead, it is well described by a double exponential model, lending support to a theoretically optimal composite Brownian walk strategy.

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Multiple route memories in an individual desert ant.

Desert ants inhabiting scrub-type environments in both Australia and Africa will travel to and from a regular site by visually guided idiosyncratic routes. It has been shown that each ant forms its own distinct and repeatable outward and inward paths. We assessed whether desert ant species Cataglyphis Velox, which inhabits a similarly cluttered environment in southern Spain, shows comparable behaviour. We found many ants quickly developed fixed routes which they follow over long periods. However, some ants exhibited two divergent paths, on either their outward or inward route (or both). Each of these paths was repeated a number of times, with use of one or other path interleaved, i.e., ants can apparently store and use more than one route memory between the same two locations. In displacement tests, they are able to recall either route when appropriate. To test models of ant behaviour, we have collected an image data-base in the actual ant habitat, with specific focus on the observed routes. This allows the development of realistic simulated environments in which to assess biologically plausible mechanisms of visual homing.

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(Re-) Adjustment of foraging site approach by desert ants, Cataglyphis fortis

Desert ants, such as Cataglyphis fortis of Saharan salt pans, Melophorus bagoti of central Australian deserts, or Ocymyrmex velox of the Namib, are individual foragers, due to their typically small and widely scattered prey items, mostly insect carcasses [e.g. 1]. We studied Cataglyphis fortis, which inhabits flat and featureless salt pans, and thus strongly relies on path integration, or dead reckoning, for navigation. According to this ecological situation, the ants do not normally refine their path integration system over time. They rather calculate homing distance and homing direction for each foraging trip independently, with the accuracy that is available from their compass and odometer readings during travel [e.g. 2, 3]. Refinement of navigation becomes possible, however, if the individual foragers exploit a plentiful and durable food source that is repeatedly visited over time. Such food sources are larger carcasses, desert plant fruits, or experimentally introduced feeding stations. In this situation, Cataglyphis adapts and optimises its approach to the feeding site. In the absence of visual landmarks, 4 or 5 visits to a feeder are necessary to establish a stable approach trajectory. Stable trajectories are characterised by fairly straight path segments and an approach into the downwind side of the feeder, where odour cues emanating from the food are used to finally localise a small and inconspicuous food source [4]. The downwind approach is adjusted, depending on nest – feeder distance, to angles deviating from a straight approach 5-8° into the downwind side [5]. What is actually learned and optimised by the ants when refining their approach trajectories, and when adjusting their approach to the particular downwind distance? We pursued this question by relocating a well-established feeding site that was approached reliably by experienced ants. The feeder was moved to the downwind side by a distance just exceeding the downwind approach distance kept by the ants. The animals thus missed the feeder on their first approach after the relocation, finding it only after lengthy searches. In this situation, the ants might either gradually readjust their approach trajectory, needing the usual 4 to 5 visits before settling into the new trajectory. This would indicate learning of the respective approach trajectories, perhaps by miniature landmarks such as pebbles or surface structures on the desert floor. Alternatively, familiarity with feeding situation and nest – feeder distance might allow rapid readjustment, perhaps already with the second visit after relocation. This would indicate a more general adjustment of a safety margin for navigation. In fact, both possibilities were observed, apparently dependent on the ants' individual foraging experiences. This indicates that experienced individuals are able to adjust their approach into a known area in a general way with regard to navigation accuracy, a result rather unexpected in short-lived foragers like desert ants. [1] Wehner R (2003) Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol A 189: 579–588. [2] Wehner R, Müller M (2006) The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. PNAS 103: 12575-12579. [3] Merkle T, Wehner R (2010) Desert ants use foraging distance to adapt the nest search to the uncertainty of the path integrator. Behav Ecol 21: 349-355. [4] Wolf H (2007) Desert ants adjust their approach to a foraging site according to experience. Behav Ecol Sociobiol 62: 415–425. [5] Wolf H, Wehner R (2005) Desert ants compensate for navigation uncertainty. J Exp Biol 208: 4223-4230.

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P 140

Distance estimation in desert ants, Cataglyphis fortis – the optic flow factor

Desert ants Cataglyphis employ path integration to return to their nest, a navigation feat that requires continuous updating of a home vector. The angular component of this vector is provided by a celestial compass (Wehner 2003 J Comp Physiol A 189: 579). Distance estimation is performed by a stride integrator (Wittlinger et al. 2006 Science 312: 1965) that accounts for stride length and walking speed. This was demonstrated by examining homing distance after manipulating the ants' leg lengths, and resulting changes in stride lengths. In these "stilts-and-stumps" experiments, slight differences in homing distance estimation were observed compared to predictions derived from altered stride lengths. Overestimation of homing distance by ants with elongated legs ("stilts") was greater by 18.5% than predicted, and short-legged ants' ("stumps") underestimation was 15.9% greater than predicted from stride-frequency-to-stride-length relationships. Ants on stilts ran too far and ants on stumps, too short, all groups searching a bit farther from the nest–feeder distance than would have been expected from the imposed changes in stride lengths (Wittlinger et al. 2007 J Exp Biol: 198). A possible explanation for these deviations would be a discrepancy in the effects that manipulation of leg length has on change of stride length and change of body height - i.e. eye-to-substrate distance, and thus ventral optic flow perception. This is true even though we tried to exclude ventral optic flow in the original experiments by carefully choosing a featureless channel set-up to minimize visual cues. Stilted ants were elevated and stumped ants were lowered down and thus might have experienced a greater change of ventral optic flow than that brought about by the change of stride length. In the present study we replicated the above stilts-and-stumps experiments. But instead of just minimizing visual cues by providing a featureless set-up we fully excluded the perception of ventral optic flow by covering the ventral halves of the ants' eyes with paint. In this situation, the ants did search for the nest exactly at the predicted homing distances. As a control, we analyzed the walking behavior (stride-length-to-stride-frequency relationship) of normal ants and ants with ventrally covered eyes. No differences in walking behavior were observed. In summary, the ants' distance estimator can work without any ventral optic flow input, relying on a stride integrator exclusively, but ventral optic flow, if present, might serve a control or calibration function.

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Memory goes crossmodal: Visual and olfactory navigation in ants

The desert ant, Cataglyphis fortis, uses both visual (Wehner 2003) and olfactory (Steck et al. 2009) cues to guide its return to its nest. This natural behavior provides us with a sensitive method for examining the time course of unimodal and bimodal learning under field conditions, which has yielded striking results. Ants are relatively slow to learn the location of the nest when it is specified by just an olfactory or a visual cue. But learning is accomplished in one single trial when visual and olfactory cues are provided together as a bimodal compound. Initially, ants trained with the bimodal compound responded as accurately to visual or olfactory elements presented singly as they did to the compound, but after 15 trials the compound evoked an accurate response, but single elements did not. This dramatic change in associative strength between elements and compound depending on the degree of experience might prevent confusion arising from environmental ambiguity and thus help Cataglyphis to navigate within visually cryptic habitats.

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521 **P 142**

Parsimonious route learning strategies in ants: A possible role for observed scanning behaviours

Studies of visual navigation have revealed how insects combine simple strategies to produce robust behaviour and insect navigation is now an established model system for investigations of the sensory, cognitive and behavioural strategies that enable small-brained animals to learn and utilise complex sequences of behaviour in the real world. We take a modelling approach to investigate the possible interactions between behaviour, learning and the visual ecology of route based navigation. An ant can only translate in one direction relative to its viewing direction, namely forward. Thus, if the current view is similar to a remembered view from a learned route, it is likely that the current viewing direction will also represent the correct direction to move in order to follow that route. We propose that if ants are able to somehow recognise familiar views, then they can follow routes by scanning the environment and moving in the direction that is most similar to the views experienced during learning. Support for such a strategy comes from behaviours observed in both desert ants and wood ants. When released in an unexpected but familiar place the desert ant melophorus bagoti scans the environment by turning rapidly on the spot. More than one scan maybe performed with short straight runs of a few centimetres separating them before the ant finally sets off in a seemingly purposeful manner. Wood ants exhibit a second form of scanning behaviour. Instead of walking in a straight line, wood ants instead tend to weave a somewhat sinuous path. This results in scans of the world centred on the overall direction of movement. We provide a proof of concept for this idea by training a classifier to recognise views and learning a series of non-trivial routes through a real-world environment using a large gantry robot equipped with a panoramic camera. We also explore how route structure affects this process and discuss how this might relate to innate behaviours such as beacon aiming.

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P 143

Moving between day and night: ant navigation in distinct temporal niches

The ability to navigate between places of significance is of fundamental importance to all animals. Depending on their lifestyle and habitat animals use diverse navigational mechanisms, although by comparing distantly related species it is hard to know what causes this diversity. We studied the navigational knowledgebase of day- and night-active congeneric, sympatric Australian ant species (genus Myrmecia aka bull ants, jack jumpers) to investigate in discrete temporal niches the adaptive shift in salience-dependent navigational strategies in ants. We tracked the paths of displaced ants with centimetre accuracy using Differential GPS to determine the extent to which day- and night-active ants path integrate or rely on landmark guidance. We find that both diurnal and nocturnal ants use visual landmark cues both while foraging and homing. Bullants do path integrate, but this information is suppressed when they are displaced within familiar landmark territory. Diurnal M. croslandi foragers are able to home directly when displaced 10-15m in any direction from the nest, clearly relying on landmark guidance for homing and revealing an unusually rich knowledge of their environment. We reconstructed the ant's scanning behaviour and the landmark panorama at the release sites in a quest to identify what exactly they are looking at before deciding on a direct route home.

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P 144

Light functions as a trigger for synaptic plasticity in visual brain centers of the desert ant Cataglyphis fortis – but can it also bias the ants` behavior?

North African desert ants (Cataglyphis fortis) undergoe an age-related polyethism subdividing the colony into dark-adapted interior workers and light exposed short-lived outdoor foragers. During daily foraging trips the ants are able to precisely return back to their nest. To accomplish this impressive navigational feat the ants use a path integration system combining a polarization compass, a proprioceptive odometer, landmark orientation (Wehner, J Comp Physiol A, 2003) and olfactory cues (Wolf et al. J Comp Physiol A, 2005). We explore neuronal plasticity during the quick transition to a light-exposed lifestyle in the mushroom bodies (MBs) - sensory integration centers involved in learning and memory, presumably including processing of landmark information. To investigate structural synaptic plasticity we immunolabeled and quantified pre- and postsynaptic profiles of synaptic complexes (microglomeruli,MG) in the visual (collar) and olfactory (lip) input regions of the MB calyx. The results show that the behavioral transition is accompanied by a general volume increase of the MB-calyx which is associated with a decrease in MG density in the collar and, less pronounce, in the lip. The results further indicate that presynaptic pruning of visual projection neurons and dendritic growth in MB intrinsic neurons (Kenyon cells) is involved. Light exposure of dark-reared ants of different ages demonstrated that the pruning effect in the collar is primarily triggered by light rather than an internal program. This effect is verified by the fact that dark-kept ants age-matched to foragers had MG numbers in the collar similar to those in interior workers. Current investigations seek to find out whether the light-dependent neuronal plasticity in visual input regions of the MBs affects the ants` behavior: can a precocious transition of interior workers to foragers be triggered by light exposure of young ants and turn them into premature foragers? Support by DFG SFB 554, A8.

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Theme and variation in the flight motifs performed by bumblebees during learning flights when leaving the nest and during approach flights on their return

Visual information about the surroundings of the nest is acquired during learning flights for use during subsequent return flights. We will describe the similarities and differences between the most prominent flight motifs observed during the two flights and suggest how these similarities and differences are adapted to the acquisition and use of visual information.

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The fine structure of honeybee and wasp homing behaviour

Honeybees and ground-nesting wasps can memorize the spatial location of important places using a visual representation of the goal environment. When the insect leave their goal location for the first time they turn to face the goal and move away from it flying sideways and backwards in a series of arcs during which they gain distance from the goal. The structure of these learning flights suggests that they serve to record the scene around the goal from defined vantage points and to acquire distance information through motion parallax. Our hypothesis is that bees and wasps actively organize the visual information they receive through series of structured head and body movements. The aim of this study is to understand the functional significance of head movements during learning and return flights, and how their organization relates to the insects' homing ability. We used three high-speed cameras to record insects approaching and departing from a goal that was either a food source in an indoor flight-arena (honeybees) or a nest in the ground outdoors (cerceris wasps). We measured the 3D-flight trajectory and the insects's body and head orientation off-line. We found that both species change their head orientation faster than their body orientation. Body yaw turns ("saccades") are in most cases accompanied by even faster head yaw turns ("head saccades") that start about 8 ms earlier than the body saccades. We find no pronounced differences in saccade velocities and amplitudes for departing and return flights. The insects thus do not generate a pivoting parallax field as had been deduced previously from the analysis of the wasps' body orientation during learning flights. Instead they generate a sequence of translational parallax fields linked by saccadic gaze changes. These behaviourally shaped optic flow fields can help the insects to segregate objects from their background and might allow them to judge the relative and possibly absolute distance of landmarks.

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P 147

The role of different landmark features for homing honeybees

Honeybees use memorized visual representations of their environment to find back to their hive and to profitable food sources. Prominent landmarks can play an important role in pinpointing the exact goal location. Several features of landmarks seem to be relevant, such as their retinal position, size and colour. However, it is still not completely understood, how honeybees extract these cues from the complex visual input they experience during flight and how these cues are combined into a spatial representation of the goal environment and used for navigation. We investigated the behavioural relevance and the interplay of different landmark features. In an indoor flight-arena we used high-speed cameras to record honeybees approaching a previously learnt food source located between three landmarks. Cue-conflicting tests were conducted to examine the role of the spatial configuration and the texture of the landmarks. We analysed the effect on the navigational performance and the flight behaviour and find that honeybees make use of the landmark's texture, if it provides them with positional information. They rely more on pattern cues of an individual landmark than on the overall spatial configuration of landmarks, if the pattern labels the landmark close to the food source and if the saliency of the pattern is high.

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Head movements during orientation flights of the bumblebees Bombus terrestris

Foraging bees and wasps shuttle many times a day between their nest and a source of nectar and pollen. Upon leaving the nest, the insects perform so-called orientation flights to ensure they can find the nest on their return. At the start of each flight the bees normally fly slowly close to the nest entrance to memorize the visual appearance of the nest vicinity. Here we present the macro-scale analysis of the orientation flights of ground nesting bumblebees Bombus terrestris. We present the characteristic speed and timing of the bees' head and body movements, and discuss the functional significance of these steering manoeuvres. The bumblebee colony was placed in open air below a table-top, and the bees were filmed with a high-speed camera from above while they were flying in the 10cm area around the nest entrance. As do other insects (Land, 1973; Collett and Land, 1975; Schilstra and van Hateren, 1998; Zeil et al, 2007; Boeddeker et al, 2010), the bumblebees turn their head and body saccadically, ensuring low retinal image speed for at least 45% of the flight time. The low, but non-zero, speed of head rotation between saccades may indicate that the head movements are visually controlled, or that the bees are compensating for the nest retinal slip. Moving the head relative to the body helps the bees to stabilize the image, and facilitates the extraction of distance information through translational flow, presumably helping them to create a 3D visual of their environment.

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Morphology of two parallel visual pathways through the anterior optic tubercle of the bumblebee

The anterior optic tubercle (AOTu) is a small neuropil in the protocerebrum of insects and a prominent projection area of visual interneurons from the optic lobe. It consists of an upper subunit of yet unknown function and a lower subunit that is part of the sky-compass system in desert locusts. While in crickets and locusts a multitude of neurons within the sky-compass system have been characterized by intracellular recordings and dye injections, the hymenopteran sky-compass has been almost exclusively studied at the behavioral level. Here we studied the morphology and the neural connections of the anterior optic tubercle in the bumblebee (Bombus ignitus). We first investigated the morphology of the AOTu in serial semi-thin frontal sections stained with Azur II. Like in other insects, the AOTu of Bombus comprised two subunits. The small lower subunit sat on the posterior lateral side of the upper subunit. Through injection of biotinylated dextran we traced two parallel pathways from the compound eye through the AOTu to the central brain. Both pathways ran via the serpentine layer of the medulla, through the lobula to the lateral accessory lobe and included branches in the contralateral AOTu. Staining patterns after injection into the lower subunit or the upper subunit, respectively, were distinctly different and showed no overlap. While dye application to the most dorsal aspect of the medulla (the target of long visual fibers from the dorsal rim of the compound eye) revealed projections only in the lower subunit of the AOTu, tracer application to the most ventral part of the medulla labeled the dorsal part of the AOTuâ€™ s upper unit. Most neurons of both pathways had a remarkable similarity to those described in the locust. Most prominently, medulla line-tangential neurons projecting to the lower subunit had extensive branches in the most dorsal area of the posterior medulla, and projections from the AOTuâ€™ s lower subunit terminated as conspicuous glomerular structures in the lateral accessory lobe, resembling polarization-sensitive TuLAL neurons of the locust.

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395 **P 150**

Honeybee navigation: Context dependent active vision strategy

Honeybees use different visual cues provided by landmarks in the vicinity of a food source to localize it. However, it remains an open question which features of the visual environment are extracted and how the information is organised and stored for later recall. Dependent on the visual context different features of the visual environment are available and have to be gathered and processed during flight for successful navigation to the food source. Since the available visual information depends, as a consequence of the closed-loop nature of the action perception cycle, on the way the animal moves, the animal may adapt its flight style to the current behavioural context to facilitate visual information processing. To test this hypothesis we analysed navigation flight behaviour. We trained honeybees to visit a feeder that was surrounded by three uniformly coloured, dark red cylindrical landmarks. We analysed their flight behaviour with respect to the position relative to the landmark and feeder constellation. In a second experiment we changed the landmark texture from solid red to the same random texture that covered the background, which made the landmarks hardly detectable in static images. The flight durations show that the bees are able to solve their navigation task without taking significantly longer. A detailed analysis of the flight behaviour reveals that the bees adapt their flight style to the new environment. To describe navigation flights quantitatively we reduce the complexity of flight behaviour by identifying finite sets of basic flight manoeuvres, which we call prototypical movements. For doing this, we, first of all, determine bee trajectories (three dimensional position and orientation of the body length axis) from the data delivered by a pair of high speed cameras and calculate local velocities for each point of time. To the resulting velocity data we apply a clustering approach that is based on the general purpose k-means algorithm and reduces clouds of similar velocity vectors to a finite set of velocity prototypes. The assignment of trajectory data to the corresponding velocity prototype at each point of time leads to the representation of the flight as a sequence of prototypical movements. This allows us to analyse flight behaviour by characterising the prototypes themselves and the spatial distribution of their occurrence. The resulting sets of prototypical movements for the bee navigation flights generally reflect the segregation of flights into saccades and intersaccadic intervals. Within the intersaccadic intervals different prototypical movements occur that depend on the position relative to the landmarks and feeder. Firstly, the prototypes describing slow forward movements occur mainly near to the landmarks and feeder while with increasing distance the forward velocity also increases. Additionally, the bees spatially vary the sideward and upward/downward movement components probably dependent on the available visual information as, for example, vertical and horizontal contrast lines between the landmarks and the background. A comparison of the prototypical movements occurring for the solid red and the randomly textured landmarks, respectively, shows a match to each other to a large extend. Nonetheless, we find differences concerning the sideward and upward/downward movement components dependent on the different environments either containing luminance contrasts or just relative motion contrasts. This suggests that honeybees adapt their flight style to the current situation in a way that may facilitate gathering the relevant information for successful navigation.

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Biological benefits of the honeybee waggle dance by computer simulation analyses

A honeybee informs her nestmates of the location of a flower that she has visited by a unique behavior called a □gwaggle dance.□h We regard the waggle dance as a good model of the □gpropagation and sharing of knowledge□h that maintains a society, and thus we attempted to reveal the effects of the waggle dance in terms of the colony□fs benefit using mathematical models and computer simulation based on parameters from observations of the bee behavior using a video camera. Video analysis showed that the information of the waggle dance contains a substantial margin of error. Angle and duration of waggle runs varied from run to run, with the range of □} 15□‹ and □} 15%, respectively, even in a series of waggle dances of a single individual. We also found that a follower that listens to the waggle dance attended to multiple dancers before her flight and that most dance followers turned away from the dancer after one or two sessions of listening. Furthermore, we found most bees in the hive did not walk for long distance. We, then, created a Markov model of bee foraging behavior and performed simulation experiments by incorporating those biological parameters. Comparison of the result of our simulation experiments to that of the previous experiment of real bees showed that our simulation faithfully expresses the actual bee behavior and showed that information transfer by the dance was important for the effective food collection. A colony in which honeybees danced and correctly transferred information made 2.15 times more successful visits to food sources compared to a colony with non-accurate information transfer and our simulations suggested that the waggle dance is effective under particular conditions.

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P 152

Integration of polarized and unpolarized light information in the central complex of the desert locust

Many insects rely on visual information when navigating through their environment. A key role in computing the direction of locomotion is ascribed to neurons of the central complex, a group of neuropils in the center of the insect brain, consisting of the central body and protocerebral bridge (Homberg 2008, Arthropod Struct Devel 37:347). Behavioral studies on mutant fruitflies showed that particular substructures of the central complex are required for recognition of visual landmarks, visual targeting in climbing motor activity, and spatial visual orientation memory. Evidence from the desert locust suggests that the compass for celestial navigation by means of the polarized light pattern of the blue sky resides in the central complex (Heinze und Homberg 2007, Science 315:995). To further analyze visual signal processing in the central complex we have performed intracellular recordings from neurons in this brain region while the animals experienced dorsally presented polarized light simulating the polarization pattern of a patch of blue sky. Additionally a green spot of unpolarized light was rotated around the animals mimicking the position of the sun. We show that identified types of polarization-sensitive neurons of the central complex in addition respond to these light spots in an azimuth dependent manner. To investigate the responses of central- complex neurons to unpolarized light more comprehensively we presented diverse stationary and moving stimuli manually or via monitors to the animals. Responses to e.g. stationary or moving bars and looming stimuli were mainly restricted to neurons with ramifications in the upper division of the central body. Our data suggest that different sets of neurons of the central complex respond to distinct visual features relevant for spatial visual orientation.

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405 **P 153**

Insect navigation: Saccade-like turns to learned visual features as a mechanism for image matching

Visual memories of landmarks play a major role in guiding the habitual foraging routes of ants and bees, but how these memories engage visuo-motor control systems during guidance is poorly understood. We approach this problem through a study of image matching - a navigational strategy in which insects reach a familiar place by moving so that their current retinal image transforms to match a view memorized earlier at that place. Analysis of the way that navigating wood ants correct their course when close to a goal reveals a significant part of the mechanism underlying this transformation. Ants followed a short route to an inconspicuous feeder positioned at a fixed position relative to a vertical, luminance edge. They responded to an unexpected jump of the edge by turning to face the new feeder position specified by the edge. Importantly, the initial speed of the turn increased linearly with the turn's amplitude. This correlation implies that the ants' turns are driven by their prior calculation of the angular difference between the current retinal position of the edge and its desired position in their memorized view. Similar saccade-like turns keep ants to their path during unperturbed routes. The neural circuitry mediating image matching is thus concerned not only with the storage of views, but also with making exact comparisons between the locations of a visual feature in a memorized view and of the same feature in the insect's current retinal image. Our results show that the neural circuitry mediating image matching is probably quite sophisticated. It is concerned not only with the storage of views, but also with making exact comparisons between the location of a visual feature in a memorized view and the location of the same feature in the insect's current retinal image.

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687 **P 154**

Where is the information for visual homing in insects' eye views of natural scenes?

Insects can return to a location of interest using only a remembered view or snapshot taken from that location. While so-called view-based homing has received much attention in recent years, it is not yet known what features of the natural world insects remember in their "snapshot". We address this question by examining images gathered from the perspective of foraging insects in their natural foraging environments. Using intentionally parsimonious models of visual homing (Zeil et al., J. Opt. Soc. Am. A, 2003) we can answer specific questions about the efficacy of using stored views for navigation. Following recent behavioural experiments that implicate the skyline as a strong visual cue for navigation (Towne and Moscrip, J. Exp. Biol., 2008; Graham and Cheng, Curr. Biol., 2009), we have investigated how skyline cues might be used by desert ants to guide their habitual routes. Wood ants live in a very different habitat which does not readily provide a skyline. In this case, we show that the pattern of open sky visible through the canopy can provide visual information sufficient for homing. The view of the world is radically different for a flying insect. We are also investigating the impact of being airborne on the cues available for visual homing.

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Long duration directed flights by Drosophila under a natural sky

When flying indoors, fruit flies often exhibit a behavior in which straight flight segments are interspersed with rapid turns called body saccades. This stereotyped flight mode has been argued to be an optimal search strategy for an animal in a featureless environment. However, observations of flies in their natural environment suggest that they are capable of long range dispersal, with individuals traveling over 10 km in a single night. How do animals cover such distances if their flight is frequently interrupted with changes in heading? In order to address this apparent inconsistency, we developed an apparatus to investigate the behavior of individual wild-type Drosophila melanogaster with the natural sky as a stimulus. Flies were tethered to a steel pin and held in place between two magnets, but they were free to steer in any azimuthal direction as in free flight. We recorded the heading of the fly using a digital video camera during half-hour trials just prior to sunset. When natural skylight is visible, flies persistently orient to a single compass direction. This flight mode represents a novel search strategy under naturalistic conditions, and differs markedly from fly behavior when the sky is not visible. Long distance migration (and persistent tethered orientation) requires course-correcting maneuvers in the face of unexpected perturbations and obstacles. Our results indicate that compass cues present in skylight are sufficient for long term course stabilization, but exactly which cues are necessary is not yet clear. Among candidate cues present in skylight are intensity and spectral gradients across the sky, as well as the pattern of polarization degree and angle. Rigid-tether fictive flight experiments in the laboratory suggest angle of linearly polarized light as a likely candidate for this course-stabilizing cue. Together, these experiments raise many questions with regard to how animals choose locomotory modes and integrate sensory signals in ethologically relevant conditions.

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P 156

Long-term recordings from polarization-sensitive neurons of the locust central complex

The desert locust Schistocerca gregaria perceives polarized light with a specialized dorsal rim area of the eye and potentially uses the polarization pattern of the blue sky as a compass cue for spatial navigation. In the brain, polarization information is passed through the optic lobe and the anterior optic tubercle to the central complex which most likely serves as an internal sky compass. Because the electric field vectors (E-vectors) of the blue sky are arranged in concentric circles around the sun, the sky polarization pattern changes during the day like the position of the sun. Therefore, the processing of polarized light information has to be time-compensated for long range navigation. To gain insight into mechanisms underlying time-compensation in the processing of sky polarization it is necessary to establish a method that allows for long term recordings from polarization-sensitive (POL-) neurons in the locust brain. Towards this goal, we used copper wire electrodes for extracellular recordings from the lower division of the locust central body (CBL). The CBL is densely innervated by polarization-sensitive neurons and was, therefore, chosen in order to establish this method. We obtained for the first time long-term recordings lasting for several hours from POL-neurons. Single units were identified through template matching combined with a cluster analysis. The units showed polarization-opponency and had wide receptive fields over a range up to 180° along the right-left meridian. Within the visual field, E-vector tuning was not constant but changed considerably along the right-left meridian. In addition, changes in response amplitude and E-vector tuning depending on the time of day were observed. Comparison of the physiological characteristics of the units with those from intracellular recordings allowed for an assessment of the recorded cell types.

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P 157

Sun Compass Integration of Skylight Cues in Migratory Monarch Butterflies

Each year, millions of monarch butterflies (Danaus plexippus) migrate from eastern North America to central Mexico. Behavioral experiments have shown that a time-compensated sun compass is used for navigating south. Although monarchs use the sun as the primary source of compass information, polarized skylight can also be exploited. These celestial compass cues must be integrated with circadian clock information, so that flight direction can be constantly corrected in tune with the daily changes in solar azimuth. As the circadian clock is well characterized in the monarch, we started to address how the perception of skylight cues and their integration with the clock are achieved at the neuronal level. With 3D-reconstructions of brain neuropils and the identification of key neurons by dye injection, we revealed all major components of a putative compass network in the central complex and anterior optic tubercles of the monarch brain. Intracellular recordings of neurons within these regions revealed tuning to specific E-vector angles of polarized light, as well as responses to the azimuthal position of unpolarized light, irrespective of its spectral composition. This suggests that the compass uses information derived from the sun itself and the sky polarization pattern, but not the skylight spectral gradient. Responses to the sun and polarized light were mediated through different parts of the compound eye and integrated in a way that created a consistent representation of sky compass cues in these neurons. Interestingly, the majority of recorded butterfly neurons shared a common azimuth tuning. Overall, the neuronal machinery of the monarch butterfly's sun compass shares essential features with other insect species, but also possesses several unique aspects. With the presented data, this species is emerging as a model for understanding the brain processes underlying long-distance migration.

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GPS tracking of Egyptian fruit bats: First evidence for large-scale navigational map in a mammal

The ability to navigate is crucial for animals, yet navigational mechanisms are poorly understood, especially in mammals. Here we report the first high-resolution GPS-tracking of bats. When GPS-tagged bats were released near their cave, they exhibited high, fast and very straight commuting flights from their cave to remote fruit-trees, and bats returned to the same trees night-after-night. Bats displaced 44-km south homed directly to one of two goal locations â€“ familiar fruit-tree or cave â€“ ruling out beaconing, route-following, or path-integration navigational mechanisms. Bats released within a deep natural crater were initially severely disoriented but eventually left the crater towards the home direction and homed successfully, while bats released at the crater-edge top homed directly â€“ suggesting navigation guided primarily but not exclusively by distal visual landmarks. Overall, these results provide the first evidence for large-scale navigational map in mammals.

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P 159

Spatial representation in the medial entorhinal cortex of the bat

"Grid cells" in the medial entorhinal cortex (MEC) are neurons which become active when the position of a locomoting animal coincides with a vertex of a hexagonal grid spanning the entire environment. These neurons are believed to be a fundamental component of mammal"s ability to navigate in space, and have recently been a major subject of experimental and theoretical work. Yet, all current electrophysiological data on grid cells have been collected only from rodents. To extend current knowledge on MEC in mammals, we conducted the first electrophysiological recordings from the MEC of a novel animal model – the Egyptian fruit bat, Rousettus aegyptiacus – navigating in a rat-style 2-D environment. Here we will present preliminary data showing the existence of grid cells in the MEC of this bat species. While these grid cells appear to be rodent-like in some aspects, they also differ in others. These data provide new insights into the nature of spatial representation in the mammalian brain and might have important implications for current computational models of grid-cell formation.

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Controlling an external object through rat hipppocampal activity

The cellular basis of the brain spatial cognitive map seems to be formed by the place cells in the hippocampus (O'Keefe, et al., Oxford: Clarendon Press. xiv, 570, 1978). The firing of different place cells responds to the different positions occupied by animals (most often rats) during navigation. Indeed, we have previously used the information carried out by the place cells' firing rate in order to reconstruct the actual position of the animal in the maze by using an algorithm. Our objective here was, to develop a cellular-brain computer interface that would allow the control of an external device (cursor or a like). We wanted to explore if we could reconstruct the rat position and trajectory based on the firing of its place cells, and also control a device that would reproduce the animal's path. In addition, we want to control the speed's device based on the power of the theta (4-12 Hz) generated in the hippocampus, and correlates with the animal's speed. In our experiments, a rat was freely navigating in an open arena. Local field potential from the hippocampus and place cells were recorded by means of chronic implants placed in CA1 region. The experiment was divided in several steps. First, we studied the animal's speed by using a tracking system that was synchronized with the acquired EEG. For tracking the rats' position, small infrared light-emitting diodes were attached to the rat's head and a video camera system was mounted above the experimental arena. In the second step, we obtained a curve that better fits to our data (the sequences of rat positions). Our recordings showed a significant correlation (coefficient of 0.75) between theta and the animal's speed, as has been described by other authors (Andrews et al., Hippocampus, 16: 785, 2006). In the third step, we developed scripts in Matlab to control the device's movement (epuck, EPFL, Switzerland). The challenges to control an external device by a cellular-based computer interface will be discussed.

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P 161

Spatial Orientation in Japanese Quails (Coturnix coturnix japonica)

To find a given location, for example a feeding area or the nest, can be based on different cues. One of several possible strategies is the use of spatial relations between landmarks. Such spatial orientation is attributed as a cognitive skill, demanding a neuronal representation of the environment. We here investigated whether Japanese quails are able to learn the relation of food trays to distant landmarks and tested the longevity of their spatial memory. Birds were trained to find mealworms in three adjacent food cups that were part of a circle of 20 such cups. The positions of the baited feeders were always constant in relation to four two-dimensional landmarks, each with a different pattern, placed in the corners of the squared test arena. Birds were trained until they showed a significant orientation towards the correct feeders. To examine whether the quails used the given landmarks for orientation, landmarks were then shifted by 90° clockwise and the birds' orientations were measured again. After a period of either three or six weeks without an experiment, the quails were tested again to examine the endurance of spatial memory. Seven quails showed significant orientation towards the baited food cups after training. After the 90° landmark rotation all birds redirected their search to the new correct direction. In later trials, some of the birds were dithering between the old and the new direction indicating the influence of other, not shifted landmarks. Two out of four quails that were tested again after three weeks and one out of three tested after six weeks still showed significant correct orientation. Our results demonstrate that Japanese quails are using spatial relationships between distinct visual cues to orientate in space. They are able to remember specific places over a long period. Further research on quails is planned to investigate the role of hippocampus for spatial memory.

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P 162

IS THERE ANYTHING OUT THERE? MAGNETORECEPTION IN HARBOR SEALS (PHOCA VITULINA)

Little is known about the mechanisms marine mammals can rely on for mid- and large-scale orientation in the open ocean, a rather featureless environment. Beside celestial information provided by the sun, the moon or the stars, marine mammals could make use of the earth's magnetic field which provides comparatively stable directional information even when the sky is overcast or when swimming in dark or turbid water. The use of the earth's magnetic field for orientation has been shown for various species including fish, amphibians, reptiles and mammals and it was also hypothesized for marine mammals. However, unequivocal evidence that they possess a magnetosensory system is still missing. In the study at hand we challenged the hypothesis that harbour seals use magnetic anomalies to guide e. g. their foraging trips. To test this hypothesis we installed a magnetic field generating coil system on the bottom of our large seawater enclosure. The seals' task was to either dive down to two homogeneously constructed coils and to answer directly at the coil carrying the anomaly or they had to dive down to one coil and had to indicate if an anomaly was present or not by answering at one out of two response targets at the water surface. All approaches were additionally conducted with the seals wearing a stocking mask blocking visual information. We observe conspicuous behaviour of the seals during experiments. Every slight or large variation in the setup or the experimental procedure is accompanied with changes in behaviour and improved performance over a short period of time. However, so far the seals' over-all-performance does not differ significantly from chance which might be explained by the task not providing detectable or relevant information for the seals.

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Vision I

522 **P 163**

Retinal Distribution of Cone Opsins in an African Cichlid

A typical fish retina contains a highly ordered mosaic of cone photoreceptor cells. Most of these cells are double cones, in which the inner segments of two cone cells are bound together. These double cones typically are arrayed in a square mosaic in which four pairs surround a single cone. Each cone cell is generally thought to contain a single opsin protein, and opsins that differ in amino acid sequence produce visual pigments that are sensitive to different wavelengths of light. The cichlid Astatotilapia burtoni from Lake Tanganyika has blue-sensitive single cones and double cones consisting of paired red- and green-sensitive cells. The cone mosaic in A. burtoni is reported to be constant across its entire retina. In the African riverine cichlid Oreochromis niloticus the picture is less clear. Measurements of cone absorbance by microspectrophotometry (MSP) indicate that adult O. niloticus predominantly have three spectral classes of cones: red- and green-sensitive double cone members and blue-sensitive single cones. However, MSP also revealed a violet-sensitive single cone. Quantitative RT-PCR shows O. niloticus expresses red, green, blue, and violet cone opsins, consistent with the MSP results. Furthermore, a recent electrophysiological study of this species reported four cone mechanisms that were chromatically opponent and corresponded to these four opsins. In the square mosaic, single cones may express either the violet or the blue opsin gene. Coexpression of these two genes in the same cone cells is also possible. In this study, we use in situ hybridization to characterize the distributions of opsins in the cone mosaic of O. niloticus. Preliminary results suggest widespread expression of violet opsin by single cones in the centers of the mosaic squares, whereas double cone members express red or green opsin. Work is underway to reveal the distribution of blue opsin in O. niloticus and determine whether it is co-expressed with violet opsin.

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Functional diversity in color vision: Evidence for sex-dependent pentachromatic color vision in Lake Malawi cichlids

Color vision plays a critical role in visual behavior. An animal's capacity for color vision rests on the presence of differentially sensitive cone photoreceptors. Spectral sensitivity measures the visual responsiveness of these cones at different light wavelengths. Four classes of cone pigments have been identified in vertebrates. For teleost fishes, the opsin genes that produce these cone pigments were duplicated to produce numerous opsin genes. Our central focus concerns large-scale variation in color vision that may result from differential expression of opsin genes. We were interested in understanding variation in the number of cones that participate in spectral sensitivity due to individual, sex, and species differences. Cichlid fishes are an excellent model system for examining variation in spectral sensitivity since they have seven distinct cone opsin genes that can be differentially expressed. To examine the variation in cichlid spectral sensitivity we used electrophysiological and opsin gene expression techniques, and empirical modeling. We found that individual cichlids utilized different subsets of 4-5 cone pigments. Our study presents the first evidence for pentachromatic color vision in vertebrates. Cichlid spectral sensitivity involved multiple cone interactions, reflecting the requisite neural processing in color vision, and the potential for extraordinary spectral discrimination capabilities. Secondly, we show, for the first time in vertebrates, that there are sex differences in spectral sensitivity and opsin gene expression. We argue that females and males sample their visual environment differently, providing a potential neural basis for sexually dimorphic visual behaviour. Finally, spectral sensitivity differed between species and correlated with foraging mode and the spectral reflectance of conspecifics, suggesting a role of both natural and sexual selection in tuning color vision.

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P 165

Visual Basics – The Tectum Opticum in Gnathonemus petersii

The mormyrid fish Gnathonemus petersii possesses an active electric sense, an adaptation to live under low light conditions. Nevertheless the visual system (VS) is also specialized by possessing bundled photoreceptors, a tapetum, and retinomotoric activity. Our aim is to understand neural processing of visual information to reveal the advantage of these adaptations. I measured visual evoked potentials (VEP) to determine flicker-fusion-frequencies (FFF) in the optic tectum (OT). FFF was found to be ~50Hz. Best response to flicker was at 15-20Hz. Data were compared to VEPs in Carassius auratus. Here FFF was ~40Hz and the best response at 5Hz. Receptive fields of visual neurons and responses to grating stimuli were also measured. Spatial resolution of tectal neurons was low and low spatial frequencies were not responded to. Stimuli > 5° of vis. angle were sufficient to drive neurons in the OT. Cells responded to grating speeds up to 30Hz. Former results suggest that the retinal structure enables G. petersii to use the fast cone pathway under low light conditions and the sensitivity-range of rods and cones is aligned. Our results support the hypothesis of a specialized retinal structure. G. petersii has a fast VS with a low spatial resolution, which might serve to filter out interferences of small objects. The new type of retinal organization enables G. petersii to use its VS under low light conditions. The advantage of the specialization might be far-field predator avoidance.

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The Advantage of a Disordered Retina

The spatial organisation of vertebrate retinae shows incredible diversity: From the crystalline retinae of teleost fish, to the highly disordered retinae of the galliform birds. Not to mention the close packed but irregularly patterned human retina. Whilst it is easily argued that close packing of photoreceptors is an optimal arrangement if one wishes to optimise the resolution of spatial sampling, it is not so easy to explain the disorder found in some retinae. It has been suggested that the disorder allows unaliased sampling of spatial frequencies higher than the Nyquist-Shannon limit (although at the expense of aliasing lower frequencies). Here, I present a different perspective. I argue that disordered retinae are suboptimal for spatial sampling and also that they are suboptimal in terms chromatic sampling. Instead, disordered retinae should be considered `near to optimal" chromatic samplers. Within the disordered regime, the highly complex patterns of optimal sampling can be closely approximated by patterns which require far less direct developmental control. This allows for simple fine tuning in response to selective pressures. Conversely, were they arranged optimally, a small change in the requirements of the visual system would require a radical change in the retinal organisation and the developmental mechanisms responsible. The disorder reflects the advantage in having a simple and highly evolvable visual system. Without such a system, inter- and intra- species communication using chromatic signals would be far more difficult. This argument is illustrated using computational models of retinal signal transmission and observations from comparative physiology.

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Duplication of long-wavelength opsins, ommatidial heterogeneity and regionalization of the compound eye of the Glacial Apollo, Parnassius glacialis (Papilionidae)

Color vision is found in various animals including insects. The standard model for insect color vision is a trichromatic based on a set of ultraviolet (UV), blue (B), and long-wavelength (L)-absorbing rhodopsins. This pattern is however often diverse especially in the species strongly dependent on color vision, such as butterflies, by duplication of opsin gene and their characteristic expression in the retina. We have extensively studied the eye of Papilio, and have found that they have three L-opsins. The existence of three L-opsins indicates that L-opsin duplication happened at least twice in the lineage ancestral to the genus Papilio. To elucidate when and how the retinal organization is developed in the family Papilionidae, we here studied the eye of a □gliving fossil□h species, the Glacial Apollo Parnassius glacialis. Parnassius is a member of the subfamily Parnassiinae, which diverged long before the subfamily Papilioninae divided into four present Tribes. We first identified four opsin mRNAs in P. glacialis. They are one UV (PgUV), one B (PgB) and two L (PgL2 and PgL3) opsins: there was no orthologs of Papilio L1. Molecular phylogeny has indicated that the ancestral L opsin is of the L2 type, which first duplicated and produced L3 before the Parnassius lineage diverged. It then re-duplicated producing L1 in another lineage ancestral to Papilio. We further identified the photoreceptors expressing these mRNAs, and found three types of ommatidia, matching the results of light and electron microscopic histology. Surprisingly, we found a set of photoreceptors coexpressing the PgUV and PgB: the spectral sensitivity of these photoreceptors were in fact broader than those expressing either one of these opsins. However, the coexpression is restricted to the ventral region of the eye. Dorsally, the expression pattern is as simple, and presumably ancestral as what has been found in other □gbasic□h species including bees, moths and some nymphalid butterflies.

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P 168

Spectral organization of the retina of Eastern Clouded Yellow butterfly, Colias erate

The compound eye of Colias erate contains three types of ommatidia characterized by red perirhabdomal pigment and a fluorescing distal pigment. The ommatidial heterogeneity is supported by the expression pattern of four opsin mRNAs in nine photoreceptors (R1-9) in each ommatidium. Here we determined the spectral sensitivity of R1-9 in each type of ommatidium to elucidate the spectral organization of the eye of C. erate by intracellular electrophysiology and subsequent dye injection. In type I ommatidia, we found that R1 was UV sensitive and R2 was blue (B) sensitive, or vice versa. In type II and III, both R1 and 2 were B and UV receptors, respectively. Previously determined patterns of opsin distribution support that the UV receptors express the UV opsin, CeUV, and the B receptors coexpress two V opsins, CeV1 and V2. The B receptors of type I have lower sensitivity at 320 420 nm, which is attributable to the filtering effects of the neighboring UV receptor and the fluorescing filter pigment found only in type I ommatidia. The R3-8 express a long-wavelength (L) opsin, CeL, in all ommatidia. We found R3,4 were always green (G) sensitive, so CeL must be a green opsin. The sensitivity of R3,4 was narrower at 440-450 nm in type II and III, which is probably due to the lateral filtering of neighboring cells. The sensitivities of R5-8 contributing the rhabdom only in its proximal half, peaked in the red wavelength region (at least 640 nm in type I and 660 nm in type III). The red sensitivities are apparently produced by the G opsin with the red perirhabdomal filters in R5-8.

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P 169

Temporal characteristics of photoreceptor cells of the Japanese Yellow Swallowtail butterfly, Papilio xuthus

Stationary and dynamic properties of sensory signals are often processed by separate parallel sensory subsystems, e.g., the magnocellular and parvocellular pathways of the primate visual system. In insect visual systems, stationary information such as color and dynamic information such as motion appear to be processed by partially segregated pathways. We investigated dynamic properties of the very initial stage of the visual process, i.e., the retinal photoreceptor cells of the Japanese Yellow Swallowtail butterfly, Papilio xuthus. The photoreceptor cells of P. xuthus show great diversity in their physiological and anatomical characteristics – seven types of spectral sensitivity, three combinations of spectral types in the ommatidia, short and long visual fibers, and different degrees of axon collateral projection in the lamina cartridge. Intracellular recording was made from photoreceptor cells. Previously recognized seven spectral types, ultraviolet (UV), violet (V), blue (B), double peak green (DG), single peak green (SG), red (R) and broad band (BB), were recognized. Seventy two photoreceptor cells were examined for their dynamic responses to random-noise and square-pulse intensity modulated light stimulation. Half maximum frequency in cross spectra showed that all receptor types responded equally up to ~20Hz, but SG, DG, and BB extended their frequency response to ~45 Hz. Response latency as measured by cross correlation peak time was shorter in SG, DG, and BB than in UV, V and B. Response dynamics to step stimulus showed a similar pattern across spectral types whether the light pulse was given in dark or in three different mean levels. The fast dynamics of SG, DG, and BB corresponds to their short and thick axons as well as the paucity of the axon collaterals within the lamina cartridges.

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P 170

Spectral sensitivity of the principal eyes of Sunburst Diving beetle larvae.

Larvae of the sunburst diving beetle, Thermonectus marmoratus, bear six stemmata on each side of their head. The frontal pair are the principal eyes for prey capture, and are among the most unusual in the animal kingdom. They form relatively long tubes with bifocal lenses, and are characterized by at least two retinas at their ends: a distal retina closer to the lens, and a proximal retina that lies directly underneath. A key question for understanding the functional organization of the principal eyes is the spectral sensitivities of their retinas. Using cloning and in situ hybridization in third instar larvae we show that the proximal retina expresses an ultraviolet sensitive opsin (TmUV II) and the distal retina a long-wavelength sensitive opsin (TmLW). To further explore the spectral sensitivity of individual cells, we used intracellular recording and neurobiotin injection. We find that cells of the proximal retina have a sensitivity that peaks in UV, around 367 nm, with its spectral sensitivity curve matching well the predicted sensitivity of a single opsin. Most dye injections led to the labeling of multiple cells, raising the possibility that these cells may be coupled through gap junctions. We also succeeded in briefly recording from a few of the smaller cells in the distal retina. Their shape and spectral response is that of a typical long-wavelength opsin with a dominant alpha band in green (approximately 530nm) and a smaller beta band. Our findings demonstrate that each retina has its own spectral sensitivity, and that this sensitivity could not be used to compensate for chromatic aberration (in which the shorter wavelength receptors are expected to be closer to the lens than the longer wavelength receptors). Instead, the spectral sensitivity distribution is consistent with our hypothesis that the bifocal lens results in separate images on the two retinas.

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Visual pigment mediated photoresponses in chromatophores

Many fish rely on changeable integumentary color patterns for visual communication and adaptation to their surrounding backgrounds. Integumentary colors are produced by pigmented cells called chromatophores, which can react directly to light stimulation by dispersing or aggregating pigment granules, suggesting that chromatophores contain visual pigments. To examine this possibility, we examined opsin gene expression patterns in skin tissues of Nile tilapia (Oreochromis niloticus) by performing RT-PCR-based tissue screening, quantitative real-time RT-PCR on tissues, single-cell RT-PCR on individual chromatophores, and in situ hybridization and immunohistochemistry to localize the visual pigment in chromatophores. RT-PCR-based tissue screening revealed ubiquitous opsin gene expression in the skin. Quantitative real-time RT-PCR of skin tissues showed that seven different opsin genes were expressed at varying levels, with Rh2aα and/or Rh2aβ opsin genes the most prominently expressed. Single-cell RT-PCR analysis showed co-expression of opsin genes in different classes of chromatophores: melanophores-LWS, Rh2b, and SWS1; erythrophores-Rh2aα, Rh2aβ and SWS1, iridophores-Rh2b and SWS1. In situ hybridization demonstrated co-localization of multiple opsin gene expression in skin tissues. Immunohistochemistry further revealed that different visual pigments were co-expressed in individual chromatophores. The light-driven motile activity of erythrophores was elicited by stimulation with 546 nm causing pigment granule dispersion and 365 nm causing aggregation. Visual pigment mediated photoresponses in chromatophores can generate distinct changes in the coloration of integumentary tissues of fishes, which maybe important for visual communication and adapting to environmental change. This research was supported by a NSERC Discovery Grant, the Canada Research Chair program and the Canada Foundation for Innovation to CWH.

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Compound eye specialisations in four species of Australian bullants, with a focus on the nocturnal Myrmecia pyriformis.

To avoid competition and reduce predation insects have evolved physiological adaptations and behavioural strategies which allow them to access new temporal foraging niches. Ants of the genus Myrmecia belong to one of the most primitive groups of ants and are known for their large eyes and visually guided hunting behaviour. Despite this, little is known about their visual ecology. By studying four closely related sympatric Myrmecia ants, we found a strong correlation between the timing of their foraging bouts and modifications of their visual systems (Greiner et al 2007). Rhabdom diameters vary from 1.3 µm in the day-active M. croslandi through to 5.9 µm in the crepuscular/nocturnal M. pyriformis. The number and size of facets also vary in a similar manner, with the day active species having fewer and smaller facets than those of the night active species. The two species which are active at night, M. nigricepts and M. pyriformis, have a light-driven pupil formed by the primary screening pigment cells, suggesting that both retain the ability to also be active during the day. We investigated in more detail the visual performance and neural adaptations in the crepuscular/nocturnal M. pyriformis. M. pyriformis leave for foraging trips during a brief burst just after sunset that is triggered by light intensity (Narendra et al. 2010) and which finishes before the end of astronomical twilight. The majority of foragers spend the entire night on nearby trees and only return to the nest during the dawn twilight. We have shown previously that M. pyriformis relies on visual cues to reach their foraging trees and thus require reliable vision for low light conditions. Here we provide both behavioural and electrophysiological evidence that foragers may be employing temporal summation, a process that increases visual reliability by integrating signals over longer periods of time. As light levels fall, foragers stop more often and for longer periods of time as they strive to collect more light. This behaviour is also reflected in their photoreceptors which have unusually slow impulse responses. Using neuroanatomical techniques, we also explore the possibility that the ants employ spatial summation of photoreceptor signals across a number of neighbouring ommatidia at the level of first order neurons in the lamina. References: Greiner, Narendra, Reid, Dacke, Ribi & Zeil (2007). Eye structure correlates with distinct foraging-bout timing in primitive ants. Curr. Biol. 17(20): R879-R880. Narendra, Reid & Hemmi (2010). The twilight zone: ambient light levels trigger activity in primitive ants. Proc. R. Soc. B. 277(1687) p1531-1538.

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Ultraviolet Visual Sensitivity in Mantis Shrimp

Stomatopod crustaceans, or mantis shrimp, possess some of the most notable visual systems know to biology. Their visual ecology has been characterized in great detail, showing that many species of this order are capable of advanced color and polarization discrimination. The photoreceptors of stomatopod compound eyes are maximally sensitive at a minimum of at least ten different wavelength of “visible” light, ranging from 400 nm to 700 nm. This ability is in part due to the presence of photostable colored filters that tune the associated photoreceptor classes. Stomatopod photoreceptors have also been shown to be maximally sensitive to at least five discrete wavelengths of ultraviolet (UV) light, between 310 nm and 380 nm, which are not detectable by the human visual system. Little is know about the visual pigments or spectral tuning mechanisms at work in these UV-sensitive photoreceptors. Preliminary molecular and spectroscopic results from several species of stomatopods suggest that multiple UV-absorbing visual pigments and filters are responsible for the surprising diversity of spectral sensitivities observed in stomatopod UV photoreceptors.

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Characterization of eyeshine in the larvae of stomatopod and brachyuran decapod crustaceans

A striking feature of the compound eyes of many stomatopod and decapod larvae is an iridescent green or blue shine over the dark retina. We hypothesize that this eyeshine serves to reduce the contrast of the opaque retina with the background light envronment and provide an effective ocular camouflage. Since the larval body is largely transparent, a reduction in the retinal contrast with the background light will aid in the overall camouflage of the entire animal. The variation in light reflected from the eyes of larvae found in different bodies of water suggest that perhaps larval eyeshine is matched to the background specctrum for a given species. The nature of the light reflected from larval eyes suggests that it is structural in origin rather than produced by a pigment. Reported here are spectral and structural results aimed at characterizing eyeshine in stomatopod and brachyuran decapod larvae.

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P 175

How to deconstruct a complex eye: Visual system evolution in Squilla empusa (Crustacea:Stomatopoda)

Stomatopod crustaceans have complex and diverse visual systems, containing several features that exist in no other animals. These unique features include a specialized ommatidial region (the midband), spectral filtering of photoreceptors involved in polychromatic colour vision, and receptor sets devoted to the detection of polarized light. The most complex type of stomatopod eye contains six midband rows, sixteen physiologically different photoreceptor classes, four intrarhabdomal serial filters, and more expressed middle-wavelength sensitive opsin genes than predicted based on physiology. Stomatopod species, however, exhibit a wide range of variation in eye anatomy and physiology. To further elucidate visual system evolution within the stomatopods, we have chosen to investigate a species exhibiting less visual system complexity than just described. Anatomically, Squilla empusa contain only 2 midband rows and no intrarhabdomal filters. Phylogenetic reconstruction of these visual characters indicates that the less complex S. empusa eye is the result of loss of complexity. Studies of opsin expression in adult S. empusa retina show that at least four opsin genes are expressed, although physiologically there is evidence for only a single visual pigment. Interestingly, larval studies found expressed opsin transcripts similar to genes in other species that are expressed in photoreceptor classes that the S. empusa retina no longer contains. These studies illustrate that in S. empusa, the loss of visual complexity proceeds more quickly at the structural level of the eye than at the level of gene expression.

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P 176

Behavioral relevance of polarization sensitivity as a target detection mechanism in cephalopods and fishes

Aquatic habitats are rich in polarized patterns that could provide valuable information about the environment to an animal with a visual system sensitive to polarization of light. Both cephalopods and fish have been shown to behaviorally respond to polarized light cues, suggesting that polarization sensitivity (PS) may play a role in improving target detection and/or navigation/orientation. However, while there is general agreement concerning the presence of PS in cephalopods and some fish species, its functional significance remains uncertain. Testing the role of PS in predator or prey detection seems an excellent paradigm with which to study the contribution of PS to the sensory assets of both groups, because such fundamental behaviors are critical to survival. We developed a novel experimental setup to deliver computer-generated, controllable, polarized stimuli to free swimming cephalopods and fish with which we tested the behavioral relevance of PS using stimuli that evoke innate responses (such as an escape response from a looming stimulus and a pursuing behavior of a small prey-like stimulus). We report consistent responses of cephalopods to looming stimuli presented in polarization and luminance contrast however, none of the fishes tested responded to either the looming or the prey-like stimuli when presented in polarization contrast.

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P 177

Polarisation Vision and Signalling in the Marine Environment

Animals that communicate with colour in the marine environment face the problem of differential spectral attenuation. That is, water absorbs and scatters light at the short and long wavelength end of the spectrum and may render some colour signals or patterns unreliable over depth or distance viewed. Polarised signals and polarisation contrast are an alternative and rich source of information under water. Our recent work supports the idea that polarised light is utilised by many marine organisms and for some may be more important than colour for information transfer and detection tasks. Here we detail aspects of the polarised light field underwater, examine intrinsic polarisation signals in vertebrates and invertebrates and describe new areas of both linear and circular polarisation vision and their possible uses. Social communication, and contrast enhancement are more likely functions for this sense underwater than navigation.

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P 178

Two eyes in one: The bizarre optics of the principal eye two of the Sunburst diving beetle larva

Almost all animal eyes follow a few relatively well understood functional plans. Only rarely is an eye discovered which is fundamentally different from known types. Here we report that the eyes of the Sunburst diving beetle larvae (Thermonectus marmoratus; Coleoptera: Dytiscidae) is one of these exceptions, with bifocal lenses which project separate images onto physically separated retinas. These larvae are highly efficient, visually-guided predators, with 6 eyes on either side of the head. Four of these eyes (E1 & E2 on each side) look directly forward and are tubular in shape. The larvae visually scan potential prey by oscillating their heads dorso-ventrally durng prey approach. The cross sections of the retinas of these two principal eyes are elliptical, leading to narrow horizontal visual fields. Furthermore, the retina is divided into distinct distal and proximal portions. To determine the function of this dual retina, we measured the location of the image within E2 in relation to its retinal anatomy and spectral sensitivity. Specifically, we measured the back focal length for green (sensitivity of distal retina) and UV (sensitivity of proximal retina). Our optical measurements show that these eyes have bifocal lenses, with two distinct focal planes. For each individual, comparing the optical data to histological sections of the contralateral eye reveals that green images of an object at infinity are focused near the distal surface of the distal retina, and the UV images are focused near the distal surface of the proximal retina. Finally we modeled the image shift to deeper retinal layers as the object distance decreases during hunting. The combination of bifocal lenses and tiered retinas raises the possibility of a functional organization of “two eyes in one.”

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The principal eyes of late third instar larvae experience a tradeoff between hyperopia and myopia.

The larvae of the Sunburst Diving Beetle Thermonectus marmoratus are visually guided hunters with two principal camera-type eyes (E1&E2) on either side of the head. These eyes are important during prey approach and capture. The visual system of the principal eyes is especially complex, with large lenses and two distinct retinas (distal and proximal). During metamorphosis the eyes are drastically reduced, and adults emerge with compound eyes. In the last days of the third, and final, instar stage the principal eyes retain their overall shape but begin to experience anatomical changes. Here we investigate if these changes influence the ability of late third instars to capture prey. To test this question we used a frame-by frame video analysis of hunting behavior to determine the hunting success throughout the last days. In addition we measured the distances from which larvae strike at their prey. Larvae were then sacrificed to quantify to what extent morphological changes influence the focusing power of the eye. One half of the head was used to gather optical data for the lenses, while the other half was used to assess histological changes of the eye, such as the size of the eye and the distance between the back of the lens to the top surface of the distal and proximal retinas. We find that within the approximately seven days of their lives as third instars, larvae's ability to hunt is optimal between days two and five, after which it declines sharply. This decline in hunting success is accompanied by a reduction in eye length, which results in hyperopia (farsightedness). Interestingly, on the last day larvae leave the water and the new air interface results in a dramatic increase in refractive power of the lens. Late larval eye changes may be a trade off between the hyperopia that is experienced in the water, and myopia that is experienced on land.

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P 180

Eye design and predation strategies in Robber Flies

Robber flies (Asilids) comprise one of the largest and most abundant families of present day insects. Distributed through all parts of the world, over 7000 species have been described. Robber flies prey on other insects and are highly successful hunters. Most species capture prey in flight, but some species capture stationary prey while they themselves are in flight. Robber flies possess apposition eyes of the “open rhabdom” type, a design typical for higher flies. Their eyes stand out due to a massive increase in facet size in the frontal eye region. This indicates an acute zone with high spatial resolution. This acute zone could be essential for the task of spotting a fast-flying prey against a background and performing a successful capture flight. The aim of our study was to investigate the eye design of different species (from different subfamilies) of robber flies and relate it to their lifestyles and hunting strategies. It is very likely that their eye design is adapted to the requirements of the habitat: hunting in open terrain requires different visual abilities than hunting in the green and dimmer habitat of the understory of forests. We investigated the optical and physiological properties of the eyes in three species of robber flies (Dioctria hyalipennis, Neoitamus cyanurus and Lasiopogon cinctus). In all three species we found a massive increase of facet size and a change of the eye’s curvature from the periphery to the frontal part of the eye. This allows very small interommatidial angles in the frontal zone, but larger angles in the periphery of the eye. Intracellular recordings from photoreceptors in all three species showed that the eyes of robber flies are exceptionally fast. In addition, photoreceptors in the frontal part of the eye have very small acceptance angles. The eyes of robber flies thus have an acute zone with high spatial and temporal resolution in the frontal area of the eye. This acute zone most likely helps the robber flies to spot their fast moving prey.

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P 181

The structural basis of sex-limited color polymorphism in the blue-tailed damselfly

In its adult stage, the blue-tailed damselfly (Ischnura elegans) is a very colorful insect. Males have a bright green thorax after emergence and become blue when sexually mature. Some immature females are violet (violacea) and subsequently turn into a male-mimicking blue morph (androchrome) or an inconspicuous olive-green morph (infuscans), while others are foxy red (rufescens) and mature to a dull olive-brown morph (infuscans-obsoleta). Previous studies have revealed that the female color polymorphism is inherited in a Mendelian fashion following a classical dominance hierarchy with androchrome > infuscans > infuscans-obsoleta. The three morphs differ in their attractiveness to males and are thought to be maintained by frequency-dependent selection through male mating harassment. While the mating system of I. elegans and the underlying genetics have been investigated intensely, the function of the female colors in intraspecific visual communication has mostly been neglected. In an attempt to elucidate the sensory basis of the male mate choice behavior, we studied the color signals of the female integument. Spectral reflectance measurements demonstrate that the thorax of violacea displays a single peak in the ultraviolet which disappears when the damselfly matures to an androchrome or an infuscans. Androchromes show two reflectance peaks, one in the blue and the other in the green spectral range. The ratio of both peaks determines whether the thorax is more greenish or bluish. Blue androchromes perfectly mimic the color of mature males. The thorax of all other females has a low broadband spectral reflectance, ranging from green to red in infuscans and from blue to red in rufescens and infuscans-obsoleta. We assume that these broadband long-wave signals are well suited to camouflage the females in their natural habitat. Light and electron microscopic sections revealed that all thorax colors are produced by pigments and a layer of nanospheres underlying the cuticle.

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P 182

Evidence for colour vision in a nocturnal Australian marsupial: the Common Brushtail Possum (Trichosurus vulpecula)

With the recent discovery of three cone types in an Australian marsupial, the fat-tailed dunnart, trichromatic colour vision in mammals is no longer restricted to primates. Not all marsupials, however, are trichromatic. The tammar wallaby has only two cone types, and thus dichromatic colour vision. To investigate the diversity of marsupial colour vision, we used immunohistochemistry and behavioural techniques to test for colour vision in the highly nocturnal common brushtail possum (Trichosurus vulpecula). Using antibodies known to label both short and medium wavelength sensitive cones in most mammals, we have so far found anatomical evidence for two cone types. The possum retina follows a basic nocturnal mammalian pattern, with a tapetum lucidum and colourless oil droplets to increase light capture, and a very low cone to rod ratio. Using a two-alternative forced choice paradigm, we were able to show behavioural colour vision. From 3000 trials, the possum showed a persistent preference towards using brightness over colours during visual discriminations. By introducing spatial information which faded over time, we were able to encourage the subject to choose between colours irrespective of brightness. These findings highlight the diversity of colour vision within Australian marsupials, with a trichromatic dunnart, a dichromatic wallaby that readily uses colour information, and now a possum which is capable of seeing colours, but prefers to use brightness over colour as a cue during visual discriminations.

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P 183

Motion perception and visual signal design in Anolis lizards

Anolis is a diverse genus of neotropical lizards that communicate with elaborate motion patterns of the head, body and dewlap. The movements at the beginning of these displays have been hypothesized to serve the role of stimulating the visual periphery of conspecifics in order to elicit their attention. We implemented a computational model of a two-dimensional visual-motion detector (2DMD) consisting of an interconnected grid of correlation-type elementary motion detectors (EMDs) (based on Zanker and Zeil 2005) and compared its responses to behavioral visual attention responses of Anolis sagrei. The model response was consistent with a number of different aspects of lizard visual response. Lizard responses were then used to fine-tune the model's temporal and spatial parameters to allow it to accurately mimic lizard motion perception. We presented this model with artificially-generated motion stimuli and determined that the optimal stimulus is an object that abruptly moves a distance of approximately 0.3 degrees of visual angle in less than 100 ms and stops. We examined field-recorded videos of displays of five species of Anolis from Puerto Rico. Four of the species used abrupt up-and-down motion patterns in the first ten seconds of their displays that matched the predicted optimal patterns. We analyzed displays carried out in moderate wind with our 2DMD model and found that the display movements stood out effectively against the visual noise of windblown vegetation. The recorded display amplitudes would most strongly stimulate viewers 1.5-3.5 m away, and are thus well designed to attract attention of conspecifics outside of the displaying animal's territory. We concluded that motion response properties of the lizard visual periphery can be modeled effectively with a grid of simple correlation-type EMDs, and the response properties of these EMDs have strongly influenced the evolution of motion patterns used in visual displays.

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P 184

A dragon"s point of view: The visual field of the Jacky dragon (*Amphibolurus muricatus*)

The Jacky dragon, *Amphibolurus muricatus*, an arboreal lizard native to southeastern Australia, relies heavily upon visual cues for locomotion through its complex terrain, the recognition of predators and the detection of prey. During territorial disputes, male Jacky dragons also perform highly stereotyped visual displays consisting of a rapid series of tail flicks, arm waves and push-ups. In order to fully understand the evolution of movement based signals, for which the Jacky dragon has become a model species, one must appreciate the visual system responsible for their reception. The visual capabilities of the Jacky dragon, or other lizards for that matter, however remain largely unknown. The introductory tail flicking component of the Jacky dragon display has been shown to be optimized for attracting attention of receivers to the motor patterns that follow, yet neither the extent of their visual field or motion sensitivity is known, making it difficult to test ecological predictions. As part of a project to describe the visual capacity of this species, we have reconstructed the extent of the Jacky dragon's visual field, developed a schematic eye model and characterized the dynamic properties of their pupil size under varying levels of illumination. These results provide the first detailed description of visual field properties in any lizard species and are a first step towards incorporating sensory constraints into the behavioral analysis of communication signals.

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Cobras and Eyes

Spitting cobras defend themselves by ejecting their venom towards an antagonist. This specialized behavior is based on the intense pain the venom causes when it hits an eye. Contact with the skin does not result in any immediate discomfort. Combined with case reports of venom ophthalmia as a consequence of encounters with spitting cobras, this led to the general believe that spitting cobras aim at the eyes of an aggressor. We investigated the way the position and presence (or absence) of eyes influences the venom "spitting" of cobras (Naja pallida and N. nigricollis).

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 P 186

Correlation between the lateralized eye use during agonistic response and its morphological asymmetry in Siamese fighting fish

Laterality of agonistic response is frequently observed at the individual-level in lower vertebrates such as fish, whereas population-level laterality is observed in many higher vertebrates. Population-level laterality can be interpreted mainly by internal factors (e.g., cerebral lateralization), whereas little is known about the behavioral mechanisms underlying individual-level laterality. Recently, it was demonstrated that some fishes has laterally-asymmetric bodies, but the relationship between asymmetric morphology and agonistic responses has been rarely examined. Here we report the correlation between lateralized eye use during aggressive displays of male Siamese fighting fish, Betta splendens, toward their own mirror image and two morphological asymmetries. Of 25 males, five exhibited significantly more leftward eye use for assessing mirror image during left displays, and eight males exhibited predominantly rightward eye use during right displays. Morphological measurement results for the craniovertebral angle and opercular area showed that the craniovertebral angle and opercular area displayed antisymmetry and fluctuating asymmetry, respectively. We found that lateralized eye use during agonistic response by each fish was associated with the craniovertebral angle, but not with the lateral difference in operculum size; lefties (left-curved body in dorsal view) showed mainly left eye use (during left displays), and righties (right-curved body) demonstrated the opposite. We suggest that antisymmetric morphologies, such as head incline, are potentially useful for studying the association between cerebral lateralization and individual laterality of behavioral responses. Further, we propose that in fish, morphological asymmetry is related to laterality in various behaviors.

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479 **P 187**

Contrast sensitivity in budgerigars to chromatic and achromatic patterns

Bird retinas contain two cone types, single and double cones. Earlier studies suggest that double cones are used for spatial vision, while single cones mediate colour vision. However, also single cones must forward spatial information if colour vision is used for detection of chromatic patterns. Indeed, studies on chicken imply that single cones are used to detect low-frequency patterns. In this study we clarify the roles of single and double cones in grating-pattern detection tasks. We tested the ability of budgerigars (Melopsittacus undulatus) to discriminate homogenous stimuli from gratings in a two-alternative forced choice procedure. We used three groups of tests; detection limit and contrast sensitivity for achromatic patterns, contrast sensitivity for chromatic patterns along the red-green and blue-green axis within colour space, and sensitivity for patterns with a modest intensity contrast (0.11, Michelson contrast) and different spectral contrasts. The detection limit of achromatic gratings is on average 12.3 cycles/degree. Contrast sensitivity for achromatic gratings reaches a highest value of 10.2 at 1.4 cycles/degree and decreases at lower and higher frequencies. Contrast sensitivity for chromatic gratings decreases monotonically at higher frequencies and is always higher for red-green, than for blue-green contrast. Low-frequency gratings (below 1 cycle/degree) providing both spectral and intensity contrast are more reliably detected than gratings with intensity contrast only. This is true also at higher frequencies (above 7 cycles/degree) for stimuli with high spectral contrast. The results confirm that double cones set the high-frequency detection limit of patterns while single cones are used primarily to detect patterns of lower frequencies. However, single cones influence the reliability of detection even above 7 cycles/degree. These results might be of great use for the understanding of how birds perceive plumage colouration and egg colour.

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P 188

Spatial resolution in the Budgerigar (Melopsittacus undulatus): comparing anatomy and behaviour

The analysis of Nissl-stained retinal whole-mounts is a common procedure to determine the topographical arrangement of the retinal ganglion cell densities and, with the optical system parameters of the eye available, to calculate a maximum theoretical value of spatial resolution. It is well known, that many birds possess several layers of ganglion cells in the densely packed areas of the retina. However, most of the studies done so far rely on differential focusing while counting ganglion cells even in the regions where up to 6 layers occur. In order to obtain more accurate numbers of the ganglion cell densities, we invented a histological procedure, which allows to use the same retinal specimen for the whole-mount and cross-sectional counts. In five eyes from 3 individuals of wild type Budgerigar parrots we found a horizontal visual streak with a central elongated area centralis, but no fovea. The mean (n=5) peak cell density was 27970 cells/mm2 (s.d. = 4226) and the maximum visual acuity was estimated to be 6.16 cycles/degree. This value is only half the visual acuity measured behaviourally. One of the possible explanations for such discrepancy is aliasing, the optical phenomenon, which can help birds to perform on behavioural experiments.

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P 189

Lateralization of obstacle avoidance in freely flying budgerigars (Melopsittacus undulatus)

We have investigated whether budgerigars display handedness in avoiding obstacles during flight. Birds were flown through a tunnel containing obstacles, and their flights were recorded in 3D using high-speed stereo cameras. The obstacle consisted of a transversely oriented cloth wall, extending from the floor to the ceiling, carrying a checker pattern. The wall presented one or two apertures, consisting of vertical slits extending from the floor to the ceiling. Expt 1: Budgerigars were flown through a single aperture of 3 different widths: 50% 25% and 12.5% of the total tunnel width. With a wide aperture, birds flew straight through the middle of the aperture, closing their wings briefly as they passed it. As the aperture was narrowed the birds displayed a bias in the direction from which they approached and entered the aperture. Some individuals approached from the left, and others from the right. Thus, birds display lateralization at the individual level when negotiating obstacles. Expt 2: Budgerigars were presented with two apertures, each 5 cm wide, separated by a central obstacle 34 cm wide. Certain individuals preferred the left-hand aperture, while others preferred the right-hand one. When the central obstacle was displaced laterally, thereby narrowing the left-hand aperture and widening the right-hand one (or vice versa), the birds showed a preference for the wider aperture. Thus, the laterality that is displayed by the birds when they choose between two apertures of equal size is overridden by a tendency to choose the wider (safer) passage when the apertures are asymmetrical. We conclude that budgerigars display lateralization at the individual level in (a) their approach during flight through a narrow aperture and (b) their choice between two apertures of equal widths. We also find that budgerigars are capable of visually discriminating aperture sizes to select the wider aperture.

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P 190

Pigeons pretectal neurons are facilitated by two depth planes of translational flow-field stimuli simulating motion parallax.

When animals move in a 3D world populated with stationary objects located at different distances they produce distinctive patterns of visual optic flow. If the animal rotates its head around roll, pitch and yaw axes, there is rotary motion of visual flow fields in the opposite direction. Since the axes of rotation pass close to the eye, the angular velocity of visual movement over the visual field is essentially uniform. However, if the animal translates by locomotion through the same space, the translational flow-fields are more complex, and objects located at different distances move across the visual field at different velocities. Optokinetic head and eye movements serve to stabilize the visual image, and while this can be accomplished for pure rotations, only one depth plane can be stabilized for translations, leaving residual movement of images of objects closer and farther than this plane. This is motion parallax, where the direction and velocities of these objects can provide information of their distance from the animal. In these experiments we recorded from neurons in the pigeon Lentiform nuclei of the mesencephalon (nLM) when large fields of different sized spots were moved in different directions and velocities. When the best whole-field motion pattern was established for a single translational plane, we then introduced a second whole-field plane to see if the firing rate could be further facilitated or inhibited. We found that many cells, preferring fast backward visual field flow, were facilitated by the second plane of dots moving at slow velocities in the original anti-preferred direction of the neuron. Other fast cells preferred forward motion and were facilitated by a second plane of slow anti-preferred direction dots. All neurons preferring slow velocities of optic flow preferred forward motion. Results are discussed in terms of multiple depth planes during forward translation, and motion parallax.

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P 191

Biological motion perception in pigeons: Global shape or local motion?

We investigate the pigeon"s ability to discriminate the direction into which a biological motion point-light walker is facing. We do that for two reasons. First, it has been shown that pigeons have great difficulty to distinguish between mirror symmetric versions of the same static object. Here, we want to demonstrate that this is not the case for biological motion displays which they can readily distinguish, even if they are mirror symmetric versions of one another. Second, we ask whether pigeons discriminate facing direction based on motion-mediated form, or whether they rather rely on local cues. Using a two-alternative forced choice paradigm in which pigeons had to peck on one of two stimuli presented on a screen, eight pigeons were trained to discriminate between a right and a left facing walker depicting either a human or a pigeon. We then tested them with non-reinforced catch trials inserted into the continuing training sessions. In Experiment 1, these catch trials were walkers played backwards. These stimuli provide conflicting cues. The global structure points in one direction while the local motion cues the other direction. Six out of the eight birds clearly chose the direction indicated by the local motion. The other two birds based their decision on the global shape. In a number of additional experiments, we presented upright and inverted spatially scrambled versions of the displays. Only the birds that had indicated to use local motion cues in Experiment 1 could handle these tasks, the other two birds responded at random. The results show interesting individual differences between pigeons. While most of the birds rely on local motion to derive facing direction, others can handle global, motion-mediated shape. Each individual bird makes a clear decision for one or the other strategy, though. Results also show that pigeons have no problems to distinguish between mirror symmetric versions of the same stimulus as long as motion is involved.

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P 192

Image-based gaze analysis in free-viewing barn owls

Attention is a process that serves to allocate limited resources to potentially important sensory information. In humans, for example, attentional mechanisms help to detect salient targets, while other, unimportant but fully visible information is disregarded. The classical pop-out effect in visual search tasks and inattentional blindness phenomena exemplify the selective nature of the human visual system. It is, however, unclear whether similar attentional processes exist in other species. We studied visual behaviour of free-viewing barn owls with a head mounted, miniaturized video camera, the OwlCam. Barn owls move their head and not the eyes in order to look at visual targets. Thus, the view of a camera that is rigidly attached to the owl"s head will stay in register with the animal's gaze. A method is described with which the full visual scene, gaze maps and fixational loci are recovered from fixational still images recorded by the OwlCam. Two owls were confronted with an extended open-field stimulus that consisted of one odd target item among a group of several distractor items. Targets were either specified by relative changes in orientation, luminance or shape. Gaze maps were analyzed according to the probability and time of fixations located at target items. The results clearly show that barn owls do look more often and longer at target items, even when no particular task was specified. This lends strong evidence to the hypothesis that attentional processes play a critical role in avian vision, too. Noticeable parallels to human overt attention mechanisms and pop-out effects are discussed.

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Contextual modulation in the visual wulst of the burrowing owl (Athene cunicularia)

Over the last few years, our group has been studying the response properties of visual wulst neurons in an owl species that is atypically active during the day, namely the burrowing owl. Here, we investigate how such neurons respond to oriented stimuli of increasing size as a means to assess the prevalence, strength and feature selectivity of classical receptive field (CRF) surround modulation. Our results are based on 169 well-isolated neurons extracellularly recorded from eight awake burrowing owls. Manual mapping was initially used to determine the location of the CRF center. All subsequent measurements were made through the dominant eye, with sinewave gratings centered on the CRF, optimized for direction of motion, spatial and temporal frequencies. The response of most cells to high contrast stimuli (97%) typically increased up to a mean stimulus diameter of 2.04º of visual angle (spatial summation peak) and decreased as the size of the grating was further expanded. Lack of suppression was found in only 5% of our cell sample. The relative drop in firing rate between spatial summation peak and maximum suppression (suppression strength) was widely distributed with a population mean of 43%. No correlation was found between CRF size and suppression strength. Maximum spatial summation at low contrast (10%) showed a median value of 3º of visual angle. Modulation of spatial summation peaks by stimulus contrast was seen in 2/3 of the recorded cells, most often increasing the size of CRF at lower contrast. Response onset latencies shortened as a function of spatial summation. In some cells we observed an increase in latency at larger stimuli sizes, usually associated with high levels of suppression. Our results demonstrate that surround suppression is an expressive phenomenon in the owl visual wulst, and suggest that it can be modeled, at least to a first-degree approximation, by the difference of Gaussians model proposed by several investigators working in the mammalian visual cortex.

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P 194

Optical Imaging of Retinotopic Maps in a Small Songbird, the Zebra Finch

The primary visual cortex of mammals is characterised by a retinotopic representation of the visual field. It has therefore been speculated that the visual wulst, the avian homologue of the visual cortex, also contains such a retinotopic map. We examined this for the first time by optical imaging of intrinsic signals in zebra finches, a small songbird with laterally placed eyes. In addition to the visual wulst, we visualised the retinotopic map of the optic tectum which is homologue to the superior colliculus in mammals. For the optic tectum, our results confirmed previous accounts on topography based on anatomical studies and conventional electrophysiology. Within the visual wulst, the retinotopy revealed by our experiments has not been shown convincingly before. The frontal part of the visual field (0° ± 30° azimuth) was not represented in the retinotopic map. The visual field from 30°-60° azimuth showed stronger magnification compared with more lateral regions. Only stimuli within elevations between about 20° and 40° above the horizon elicited neuronal activation. Activation from other elevations was masked by activation of the preferred region. Most interestingly, we observed more than one retinotopic representation of visual space within the visual wulst, which indicates that the avian wulst, like the visual cortex in mammals, may show some compartmentation parallel to the surface in addition to its layered structure. Our results show the applicability of the optical imaging method also for small songbirds. We obtained a more detailed picture of retinotopic maps in birds, especially on the functional neuronal organisation of the visual wulst. Our findings add to the notion of homology of visual wulst and visual cortex by showing that there is also a functional correspondence between the two areas.

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P 195

Neural response to naturalistic optic flow in the zebra finch

Depth perception by stereoscopic vision is impossible for animals with laterally placed eyes and a small interocular distance, like the zebra finch. To get a reliable estimation of distance, they need to compare image differences from two different points in time instead of comparing images from two eyes at the same time point. In other words, the avian visual system analyses motion parameters such as speed and direction to derive depth information from the optic flow. The accessory optic system provides optic flow based feedback about direction and velocity of the birds self motion. The movement of the eyes which goes along with locomotion, leads to a characteristic motion pattern which affects the whole visual field. For example, straight locomotion causes an expanding optic flow pattern with the focus of expansion in heading direction. In parallel, the tectofugal visual system processes object related motion. It analyses the motion pattern from the images of single objects in the visual field and derives object motion. Recent studies, however, suggest that whole field motion is somehow integrated into object motion for better object detection. We examined response characteristics to different types of motion stimuli recorded from neurons in the nucleus rotundus, the thalamic station of the tectofugal visual system. Neuronal responses to simple self motion and object motion stimuli were similar to what is known from the pigeon. Self motion stimuli appeared to have a much stronger habituation effect on these cells than object motion stimuli. Rotundal neurons also responded to complex naturalistic stimuli, resembling a flight through a test arena in the bird's perspective. Such stimuli were constructed from head position and orientation data which were acquired in an earlier behavioural study. We present here our approach to understand the complex neuronal activity induced by this naturalistic stimulation by comparing it to optic flow parameters extracted from the naturalistic scene.

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Acoustic communication

459 **P 196**

Complex networks and sound radiation in tree crickets

Male tree crickets sing by tegminal stridulation. One wing bears a file, while the other has a plectrum. The file-bearing wing (FBW) is usually on top, and overlaps the plectrum-bearing wing (PBW). Males Oecanthus henryi generate a low frequency pure-tone in the range 2.3-3.3 kHz. In their natural habitat they undergo temperature fluctuations that affect the carrier frequency (cf) of their calls. Between 18-28°C, cf can increase by ~1kHz. Previous authors hypothesized that localized wing resonators were responsible for this temperature-dependent frequency shift. We tested this hypothesis using 3 approaches: 1. Wings movements were monitored with a motion detector synchronized with a condenser microphone. 2. Wing vibrations from actively stridulating males were recorded using laser Doppler vibrometry and acoustic recording. The vibrations of wings set still in stridulating position were measured in response to acoustic stimulation. 3. Free wing vibration: wings of mounted individuals were extended, stimulated with sound and laser scanned. Results show that 1. the tooth strike rate equals cf, both changing proportionally with temperature. 2. Free vibration from singing insects shows little variation in tuning among different cells of the same wing, but do so across wings, FBW exhibiting higher resonances than PBW. In both situations free wing resonance increases with temperature. Remarkably, as wings engage for stridulation, resonances of all cells converge to a single value, equal to cf. 3. Wing vibrations of mounted males are consistent with those of freely singing males. Mirror and harp cells show maximal deflection at all temperatures. Results do not support the original hypothesis as wing tuning and speed, and tooth strike rate all change proportionally with temperature. The tree cricket stridulation system may thus rely on a network of coupled oscillators, where the synchronization of individual resonators results in coherent sound radiation.

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623 **P 197**

Matching sender and receiver: Poikilothermy and frequency tuning in tree crickets

To communicate in noisy and crowded environments, senders often restrict signals to a narrow part of the available signal parameter space and the receiver tunes itself to this part, effectively filtering out irrelevant noise. Field cricket males restrict the frequency of calling song and female ears resonate at species-specific frequency. Tree cricket males (Oecanthus henryi) however do not restrict the carrier frequency of their calling song which varies between 2.3 and 3.3 kHz between 17 and 27°C [1]. Hence, female tree crickets are faced with the problem of efficiently responding to a calling song subjected to temperature-driven frequency changes. This problem may be solved in several ways: 1. by changing tuning with temperature to maintain a match with the male calling song, 2. by being broadly tuned throughout the frequency range of calling songs of their species, or 3. by not being tuned to any frequency. In order to understand whether females are at all frequency selective, we investigated female behavioural responses to male calling song at three temperatures (18, 22 and 27°C). The calling song was manipulated to make the temporal pattern appropriate to the temperature during the test but song frequency was varied from 1.5 to 8.5 kHz. Females phonotactically responded to a range of calling songs, several kHz wider than its natural range, at all temperatures. In order to understand the origins of this broad tuning, the mechanical response of the female ear in response to acoustic stimulation was tested at the same temperatures using the non-invasive technique of laser Doppler vibrometry. Surprisingly, female tree cricket ears were not resonantly tuned and responded coherently and nearly equally to an even wider range of frequencies than the behavioural range [2] at all temperatures.

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P 198

Temporal tuning of an identified auditory neuron: contributions of afferent timing and intrinsic properties

Crickets communicate using acoustic signals with stereotyped rhythms, and temporal pattern is a crucial cue for signal recognition. Earlier work in our laboratory showed that the temporal selectivity of an identified interneuron early in the auditory pathway, ON1, matches the temporal structure of species-specific signals. Here, we show that both the intrinsic properties of ON1 and the timing of its afferent inputs contribute to temporal selectivity. Each ear of Teleogryllus oceanicus contains approximately 65 auditory receptor neurons, about 25 of which are both tuned to the sound frequency used for intraspecific communication (4.5 kHz) and are anatomically well suited for providing direct input to ON1. Using an information-theoretic approach and modeling, we show that individual receptors are poorly tuned to the range of amplitude-modulation frequencies that occur in communication signals, but that encoding of the stimulus envelope by populations of receptors resembles that of ON1. This suggests that information carried by different receptors is pooled postsynaptically. We studied the intrinsic coding properties of ON1 by injecting randomly varying depolarizing current through an intracellular microelectrode. Coding of injected current waveforms was poorer than that of sound stimuli, and spanned a broader range of AM frequencies. However, best coding of both stimulus types occurred over a similar range of AM frequencies. Work in other systems has shown that potassium currents play important roles in regulating the spiking patterns of neurons. Application of 4-aminopyridine, which blocks low-threshold K currents, severely degrades temporal coding. By contrast, intracellular iontophoresis of BAPTA, which suppresses Ca-activated K currents, has only minor effects. Our results demonstrate that factors both extrinsic and intrinsic to ON1 contribute to its selective encoding of behaviorally relevant temporal patterns.

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Competition for call frequencies and the evolution of sensory adaptations in a tropical cricket

When insects vocalize at the same time and location, the background noise level increases, the signal-to-noise level decreases and the detection and discrimination of acoustic signals is impaired. Therefore, the communication channel is a limited resource, which results in competition for call frequencies within this channel. Here we test the hypothesis that species in habitats with high species diversity, like tropical rainforests, will be more strongly affected than those in habitats of low diversity. One possible outcome of such competition is an increase in the selectivity of tuning to species-specific calling frequencies, which we examined in the tropical cricket Paroecanthus podagrosus (Eneopterinae; Gryllinae). Consistent with our hypothesis, the ear´s tuning is significantly higher compared to the field crickets G. campestris and G. bimaculatus, where no such competition exists. We can further demonstrate that such increased tuning results in a significant increase in the signal-to-noise-ratio in the perception of the conspecific signal under noisy habitat conditions. In addition to the tuning of the ear´s sensitivity, directional hearing in crickets is also sharply tuned to a narrow range of frequencies. In European field crickets, there exists a considerable mismatch in the tuning of directionality and sensitivity. Such mismatch would be particularly detrimental for mate finding in the rainforest cricket community. We therefore examined both tunings in the same individuals of Paroecanthus podagrosus. In this species, the sensitivity and directionality tuning is driven towards the same frequency (matched filters). We therefore conclude that both the increased sharpness of hearing sensitivity and the match of both filters are highly adaptive for this rainforest cricket, and likely an evolved consequence of the enormous competition for call frequencies in the nocturnal rainforest.

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P 200

Frequency processing in the auditory system of a bush cricket

In the bush cricket Ancistrura nigrovittata processing of the male signal in the auditory pathway is followed from the sensory organ in the ear to the second level of auditory processing in the brain. From 37 sensory neurons in the crista acustica approximately the second quarter excite the ascending neuron 1 (AN1), even though single sensory neurons (cells 8 or 9) have frequency tuning corresponding closely to behavioural tuning of the females. AN1 receives frequency dependent inhibition to reduce sensitivity especially in the ultrasound, but also at low sonic frequencies. The first identified local brain neuron in a bush cricket (LBN1) overlaps with some of AN1 terminations in the brain. Its frequency tuning and intensity dependence strongly suggest it to be directly postsynaptic to AN1. Spiking in LBN1 is only elicited after summation of EPSPs evoked by individual AN1-action potentials. This serves as a filtering mechanism reducing the sensitivity of LBN1 and also its responsiveness to ultrasound as compared to AN1. As a consequence, spike latencies of LBN1 are long (ca 30 ms) despite it being a second order interneuron. This is surprising given that the female reply to a male song has about the same latency. Additionally, LBN1 receives frequency specific inhibition further reducing its responses to ultrasound. This demonstrates that frequency specific inhibition is redundant in two directly connected interneurons on consecutive levels in the auditory system of this bush cricket.

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P 201

Variability in the male's calling song correlates with variability in phonotactic and neuronal responses in female cricket Acheta domesticus

Under controlled laboratory conditions at temperatures between 22-24 °C, males produced calling songs with syllable periods ranging from 46.2 to 80.0 ms with a mean of 64.9 ms. Raising the temperature shortens the mean syllable period by approximately 2.0 ms/°C, and lowering the temperature lengthens the mean syllable period at the same rate. During phonotaxis at 22-24 °C, females respond to calling songs with syllable periods ranging from 30-90 ms. On average, the most attractive syllable periods are 60 and 70 ms. This correlates with the mean syllable period produced by the males at 22-24 °C. As the temperature increases, the most attractive syllable period decreases at a rate of approximately 2.5 ms/°C, and a decrease in temperature increases the most attractive syllable period at the same rate. Though the syllable periods produced by the males and responded to by the females change at a similar rate with a change in temperature, the females' responses are broader than the range of syllable periods the males produce for a given temperature. One possible explanation for this phenomenon is that in the field, a female may be at a different temperature than a singing male. To avoid missing a potential mate because of this temperature mismatch the female responds to a wider range of syllable periods at a given temperature than those produced by the males. The L3 neuron is a first order prothoracic interneuron in female crickets that responds selectively to the syllable period of the male's calling song. It has been proposed that the L3 neuron is responsible for syllable period-selective responses during phonotaxis. Preliminary data shows that L3s' syllable period-selective responses are temperature-sensitive. As temperature increases, L3s' peak in syllable period-selectivity shifts to shorter syllable periods, at a rate of approximately 3 ms/°C. This shift in the L3s' syllable period-selectivity correlates with the temperature-induced shifts in the females' phonotactic responses to the male's calling song.

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The potential neuronal basis of leader preference in the chorusing bushcricket Mecopoda elongate

The calling song of the bushcricket Mecopoda elongata consists of regularly repeated chirps, which, in a chorus situation, are imperfectly synchronized with those of other males. Some males produce their chirps earlier (leaders) compared to others (followers). In two-choice tests females exhibit a strong preference for the leader signal of two overlapping chirps (Fertschai et al., 2006). Römer et al. (2002) argued for a neuronal bias favouring the representation of the leader signal in a pair of local interneurons with mutual inhibitory interactions (omega-neurons). Since the decision for a leader signal most likely occurs in the brain, afferent neurons need to be postulated with a leader-biased response. As a likely candidate interneuron that encodes individual syllables of conspecific signals we investigated the directionally sensitive TN1-neuron. We recorded the spiking activity of this neuron simultaneously in both neck connectives under various stimulus conditions simulating chorus situations, varying distances between sender and receiver, as well as background noise levels. A strong contralateral inhibition results in a suppressed response of the ipsilateral neuron to the follower signal, thus creating a bias favouring the response ipsilateral to the leader site. For this response bias, the time difference between leader and follower chirps has a stronger impact compared to the sound level of the follower chirp. Reliable responses to conspecific signals were found up to distances of about 10 m. When conspecific signals were presented together with natural background noise that was recorded in the tropical rainforest, the response to conspecific signals was slightly reduced. These results suggest TN1 as a candidate interneuron that informs the brain about a leader bias, which appears the important information for the female choosing among various males. Fertschai I. et al. (2006): J Exp Biol 210: 465-476. Römer H. et al. (2002): Eur J Neurosc 15: 1655-1662.

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Selective auditory processing of conspecific male cricket calls by female Gryllus bimaculatus: prothoracic auditory neurons are causally involved in controlling selective phonotaxis

Individual female G. bimaculatus make substantially different choices for the syllable periods (SPs) of model calling songs (CSs) they respond to phonotactically. Females most commonly included the SPs found in the conspecific male's CS. Variability in the choices different females, or the same female tested serially, was usually in response to SPs outside of, or at the boundaries of the conspecific range. For females that responded to a wider range of SPs, nanoinjection of either juvenile hormone III (JHIII) or picrotoxin into the prothoracic ganglion immediately narrowed their range of SPs. In searching for prothoracic auditory interneurons that responded selectively to the SPs of model CSs, only the AN2 neuron exhibited selective responses that paralleled the female's phonotactic choices. Like the females' phonotactic choices for SPs that were outside of the conspecific range, the AN2 neuron's selective processing exhibited substantial plasticity in responding to these same SPs from female to female. Evaluation of AN2's responses to model CSs with different SPs demonstrated SP-selective processing by AN2's in large numbers of females that was very similar to SP-selective phonotaxis. We further demonstrated that both JHIII and picrotoxin, when nanoinjected into the prothoracic ganglion, substantially impact the SP-selective processing of the AN2 neuron. Since both selective phonotaxis and selective processing by AN2 exhibited substantial plasticity from female to female, we next evaluated AN2"s selective processing and the female's selective phonotaxis in the same females. There was a highly significant correlation ($p<.0005$) between the SP-selective responses of the AN2 neuron and the phonotactic choices made by the same female. The clear effects of JHIII and picrotoxin on the SP-selective processing by the prothoracic AN2 neuron and on SP-selective phonotaxis strongly support SP-selective processing in the prothoracic ganglion as a causal step in the female's SP-selective processing that leads to SP-selective phonotaxis.

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Brain neurons for auditory processing and phonotaxis in the cricket

Although cricket phonotaxis has been a model system for auditory processing in insects for almost 50 years, we still have no proper understanding of auditory circuits in the brain controlling this behaviour. Analysing the activity of brain neurons in crickets actively performing phonotaxis appears to be a promising approach. We therefore record auditory brain neurons with glass microelectrodes in tethered females of G. bimaculatus that walk on a trackball and steer towards the male calling song. This allows a functional analysis of the system by (1) identifying the morphology of the neurons, (2) comparing their activity patterns with the auditory steering behaviour and (3) testing the functional significance of neurons for phonotaxis by intracellular current injection. We identified several types of local brain neurons that exhibit dense arborisations around the projections of the ascending neuron AN1 and form a ring-like auditory neuropil in the brain. Some of these local neurons receive excitatory and inhibitory inputs and copy the pulse pattern of the male calling song. Other neurons are tuned to the pulse repetition rate and may be involved in the process of pattern recognition. Two local neurons project contralaterally linking both auditory neuropils in the brain and may support pattern recognition and steering in split song paradigms. At least one neuron responds with a very short latency of 20 ms, and seems to project to a region where descending premotor interneurons originate. This may indicate processing of directional information parallel to pattern recognition networks. Finally our stainings provide some preliminary evidence for a descending interneuron that may receive its inputs directly in the auditory brain neuropil. Current experiments focus on indentifying the structure and response patterns of this interneuron.

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P 205

Unexpected variation in the calling song and phonotaxis of two field cricket species (Gryllus veletis and G. pennsylvanicus)

Recent phonotaxis experiments with Gryllus veletis and G. pennsylvanicus, showed more variability in the females' response to model calling songs than was expected at 20-24 ºC (Stout et al. 2010). Some females were very unselective, responding to model calling songs with syllable periods outside the range reported for males' calling songs, and many crickets responded to some of the calling songs within an attractive range but not to others (termed "skipping"). In a field study, we examined the extent of the variability in the two species' calling songs to help determine potential causes for the variation in the females' response. The variability observed in both species' calling songs was greater than previously published. But, at 20-24 ºC, the range of syllable periods produced by the males was still less than the range to which unselective females responded. Additionally, most of the temporal features of the calling song that are repeated within in a chirp (syllable duration, syllable amplitude, and inter-syllable interval) increased in value as the chirp progressed, and they were positively correlated with each other. We hypothesized that some of the variability and "skipping" by females might have resulted from using "static" model calling songs, which had constant values for all the repeated features within the chirp. To test this hypothesis, we are evaluating phonotaxis using "dynamic" model callings songs that incorporate the increasing values and internal covariation of features within the chirp observed in field recordings. At the population level, preliminary data do not indicate a female preference for any one of the dynamic model calling songs, and skipping occurs at the same frequency as observed in response to static model calling songs. The values for each feature in the dynamic model calling songs were based on population-wide values, which may have not reflected an individual male's calling song accurately. We are now revising the model calling songs to more accurately reflect the individual calling songs recorded in the field.

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A male you can count on: The complex call of male Scudderia pistillata and the acoustic tick response of the female

In dueting katydids (Orthoptera: Tettigoniidae: Phaneropterinae) both the male and female emit acoustic calls for mate attraction. In the broad-winged bush katydid, Scudderia pistillata, the male call is a unique counting sequence. Analysis of over 500 male bouts shows a stereotypic sequence of adding one or two pulses to each subsequent phrase in a bout until they reach a plateau around 9 pulses per phrase. Possible explanations for counting are discussed, including male rivalry, increased active sample space of the call and female preference for more pulses. In response to male calls, female dueting katydids give an acoustic tick a specific time after the male call. Female response to playbacks of differing series of pulses was performed. Analysis of female tick response shows that females produce more ticks to male phrases with more pulses. Whether tick number relates to mating success for males that produce more pulses is currently underway. Preliminary analysis shows the sequence of phrases does not affect female response. Experiments on the effect of previous phrases on the subsequent response of the female are also currently underway.

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FFT for the flies: Songs and ears of Drosophila

The Drosophila may be small, but the range of behaviours it exhibits has been keeping scientists busy for decades. Here we investigate the relationship between the spectral compositions of the fliesâ€™ courtship songs and tuning of their antennal ears. Using the standard technique, we recorded courtship songs of the seven closely related Drosophila species. The songs typically consisted of a periodic sine-like component, and trains of short (~ 10ms) high-frequency pulses. Common problems of the spectral analysis of the pulse trains are the broadness of the FFT lines and the irregularity of interpulse intervals within the train. To overcome these problems, we developed a new Pulse Rearrangement method, which proved to be highly successful in producing spectra of single pulses. In addition to song recordings, we used Laser-Doppler vibrometry to record free fluctuations of the fliesâ€™ antennal ears. These free fluctuations are well described by a harmonic oscillator model in all species and can be used as a simple tool to determine the earâ€™s frequency response properties. The peak frequencies of the ear tuning is strongly correlated with the spectral composition of the courtship songs, so that the species that have higher frequency pulses in the songs, also have ears tuned to higher frequencies. The mechanical responses of the ears to the artificial and natural stimuli (such as wingbeats) can shed some light on the advantages and origins of this intriguing sensory tuning.

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P 208

Auditory temporal resolution of the cicada Tettigetta josei

Mate finding in the cicada Tettigetta josei is mediated by acoustic communication. The species-specific male song is composed by complex phrases with the shortest pulse periods around 4 ms. We investigated the ability of the nervous system to resolve the fine time structure of the song and its dependence on temperature. Intracellular recordings of ascending auditory interneurons and simultaneous auditory nerve recordings were obtained while the body temperature was controlled with a Peltier element in the range 16-28 ºC. The experimental stimuli were the species' calling song pulses presented at different rates. A total of 27 cells, each at several temperatures, were recorded. Temporal resolution was evaluated using periodogram, vector strength and averaging analyses. The interneuron responses were classified into 3 groups: a first group sensitive in the high frequency range (14-20 kHz) around the calling song peak, and two other groups, responding both to lower (3-6 kHz) as well as high (14-20 kHz) frequencies, separated by the presence or absence of inhibitory input. Group 1 (high frequency only) exhibited higher resolution throughout the tested range (8 ms at 16 ºC, 4 ms at 24 ºC) (n=4). Group 2 showed the worst temporal resolution (16 ms at 16 ºC, 6 ms at 28 ºC) (n=4). Group 3, where inhibitory input occurred, presented intermediate performance, resolving 4 ms at 28 ºC (n=19). Thus, the shorter song pulse periods can be resolved at least from 24 ºC (group 1). The auditory periphery does not seem to be a limiting factor as averaging analysis of whole auditory nerve recordings shows that the pooled receptor response is able to resolve gaps of at least 1-2 ms in the whole temperature range tested. Further studies will be necessary in order to verify if the temporal resolution measured in the ascending interneurons, and thus available in the brain, is actually used in behavioural decisions.

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Getting over background noise: Mechanisms underlying acoustic communication in adverse environments

Biotic and abiotic environmental noise is ubiquitous in the habitats of sonically communicating organisms and can mask vital intraspecific vocalizations. The risk of masking is contingent on the frequency and temporal overlap between the signal and the ambient noise. As a result, some species have evolved to place their calls within "silent windows" of their environment. The Southeast Asian frogs Odorrana tormota and Huia cavitympanum call adjacent to rushing montane streams and waterfalls that produce high-intensity sound spanning the human audible spectrum (ca. 20 Hz – 20 kHz). Both species communicate with ultrasonic frequencies (> 20 kHz), making them the first non-mammalian vertebrates shown to do so. We hypothesize that these distantly related species converged on ultrasonic communication to improve the signal-to-noise ratio of their calls amidst the broadband background noise of their habitats. We used histology, immunohistochemistry and confocal microscopy to compare the morphology of the peripheral auditory systems of O. tormota and H. cavitympanum, which have upper hearing limits of 34 and 38 kHz, respectively, with those of Rana pipiens, a species with an upper detection limit of ca. 3 kHz. Our results suggest that the ultrasonic species have converged on critical, small-scale functional modifications of the auditory periphery that subserve high-frequency detection. These data present the intriguing possibility of convergence in the communication behavior and underlying sensory physiology of frogs that have evolved in environments characterized by broadband background noise. However, we will also discuss the apparent rarity of ultrasonic communication among amphibians, which raises interesting questions regarding the ecological and physiological constraints that may prevent the widespread use of this communication strategy.

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 P 210

Processing of male's calls embedded within a chorus by female gray treefrogs, *Hyla versicolor*: Behavioral and neurobiological measures

During the reproductive season, male *Hyla versicolor* produce advertisement calls to attract females. Females exhibit phonotaxis and approach the individual callers, resulting in amplexus. For frogs that call from dense choruses, the extent to which and the range from which a male's advertisement call within a chorus can be heard by a receptive female leading to phonotaxis is unclear. In the natural habitat, frog calls propagate through the forest environment, and are attenuated by and interact with the atmosphere, the forest substrate, and foliage; the fine temporal structures of calls crucial for species recognition and mate selection are degraded as a result. We conducted a series of field and laboratory phonotaxis experiments to investigate female's responses to chorus recordings made at increasing distances from a calling male, as well as to synthetic conspecific and heterospecific calls. We also investigated the effects of signal attenuation and temporal degradation on phonotaxis through acoustic playback experiments with synthetic and naturalistic stimuli. Females showed directed orientation to choruses in the field at distances up to 100 m. In contrast, in the laboratory, females oriented and were attracted to chorus sounds from 1 m to 32 m only, but not from > 32 m. Degradation had a more profound impact on phonotaxis than attenuation. We further investigated signal representation in the auditory midbrain via single-unit extracellular recordings. Comparison of responses to chorus sounds and SPL-matched artificial conspecific calls showed that the synthetic stimuli elicited stronger responses regardless of the SPL. While a sub-population of cells (less than 36%) showed activity to distant (>32 m) stimuli, the majority of cells responded to chorus recordings from 1-32 m. This correlates with our behavioral results. For most units, the synthetic con- and heterospecific calls elicited the highest spike counts.

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P 211

Physiological correlates of variation in mate choice in a neotropical frog - or why the frog lost its squawk

We compared auditory sensitivity of Engystomops petersi frogs that differ in male advertisement calls and female preferences. Males of this genus have a simple call, the whine, that primarily excites the amphibian papilla (AP). Males in some clades add a second call component, termed a squawk in E. petersi, with maximal energy in the frequency range of the basilar papilla (BP). For example, males at Yasuni (Y) produce whines and whine-squawks, whereas males 20 km away at La Selva (LS) produce only whines. LS females show no preference for the squawk, and Y females prefer whine-squawks. We recorded the masked auditory brain stem response (mABR) in LS and Y frogs using three subdermal needle electrodes placed dorsal to the eardrum and brainstem. We stimulated the frogs with impulses and tones from a Beyer 48A headphone in a coupler, sealed over the frog's eardrum with Vaseline. The general shape of the audiogram is bimodal, with peaks below 1000 Hz (AP) and above 1400 Hz (BP). mABR thresholds are approximately 60 dB at the AP peak and 70 dB at the BP peak, with a region from 1300-1400 Hz of very low sensitivity. The audiograms show consistent differences both between the two types and between sexes. The most striking difference was the BP sensitivity at 1600-1800 Hz in the Y females that was absent in Y males as well as in both LS males and females. The difference in auditory sensitivity of females matches the behavioral differences in preferences for whine-squawks. We propose that the behavior of LS females is caused by lack of sensitivity to the frequencies of the squawk. Frogs in the genus Engystomops generally show BP sensitivity around 2 kHz. Therefore, the condition of the LS frogs is likely derived, i.e. these frogs lost the ability to detect the squawk. It remains to be seen whether the change in auditory sensitivity preceded the change in calling behavior or vice versa, but to us, an initial loss of auditory sensitivity is the most straightforward hypothesis.

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Frustration in Synchronized Calling Behavior of Japanese Tree Frogs

Synchronization has been observed in various biological systems; for example, flashing of a firefly swarm and calling of crickets. Moreover, mechanisms to realize such cooperative behavior are mathematically studied, including a phase-oscillator model where each element is assumed to behave periodically and interact with the others. In this presentation, we focus on talk about frustration phenomena experimentally observed in synchronized advertisement-calls of Japanese tree frogs (Hyla-japonica) and its plausible mathematical modeling. While the single male Japanese tree frogs call nearly periodically, they can hear sounds through their eardrums. Therefore, the males can interact by producing and hearing sounds, and calling behavior of several males is understood as a system of coupled phase-oscillators [1, 2, 3]. First, we recorded calling behavior of two male Japanese tree frogs with two microphones, separated sound signals of each frog by ICA methods, and confirmed robust call-alternation or almost anti-phase synchronization [1]. Second, we proposed mathematical model with two coupled phase oscillators representing experimental results of call-alternation by two frogs, extended the model to a system of three frogs, and theoretically predicted the occurrence of various synchronization phenomena, such as 1:2anti-phase synchronization and tri-phase synchronization [2]. Note that calling behavior of three male Japanese tree frogs is frustrated: namely, while almost perfect anti-phase synchronization is robustly observed in a system of two males, three frogs cannot synchronize in anti-phase. Finally, we experimentally investigated spontaneous calling behavior of three frogs and observed various types of synchronized behavior [3]. [1] I.Aihara, Phys. Rev. E 80, 011918 (2009). [2] I.Aihara, K.Tsumoto, Mathematical Biosciences 214, pp.6-10 (2008). [3] I.Aihara et.al. Complex and Transitive Synchronization in a Frustrated System of Calling Frogs, in preparation.

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557 **P 213**

Strategies in confronting interferences in anuran sound communication

Animals communicating by sound employ different strategies to overcome noise interference from biotic and abiotic sources. Anurans, as highly active vocal vertebrates, are particularly affected by such intrusions; when confronted with prolonged noise of various origins, they respond by either increasing or decreasing their vocal output. On the other hand, interference from acoustic signals among different vocally active species has been subjected to restricted study. The acoustic environments in temperate latitudes are relatively simple; few anuran species vocalize in synchrony, in contrast with the typical jamming of tropical sound landscapes. To gain an understanding of the abilities of anurans from temperate environments to overcome interferences of various origins, we explored the vocal activity of frogs Batrachyla in the southern temperate forest in Chile during exposures to prolonged natural abiotic noises of wind, creek and rain, to a band□pass noise centered at about the dominant frequency of their calls (2000Hz) and to heterospecific calls. In response to prolonged noises, males of Batrachyla taeniata and B. antartandica increased their vocal output significantly, while B. leptopus did not alter its calling rate. When presented with heterospecific calls, the three species decreased their vocal output as compared to their responses to conspecific signals (GLM ANOVAs and Tukey post□hoc tests, $P < 0.05$). Single□unit electrophysiological recordings of midbrain auditory neurons show correspondences between these behavioral responses and the ability to sustain neuronal discharge in the presence of noise. The different strategies exhibited by these species in the presence of prolonged noises prompt to further explorations on the adaptive significance and evolutionary determinants of such vocal strategies. The different responsiveness to temporaly□structured heterospecific signals and continuous noises within a species point to different mechanisms of vocal activation in these vertebrates. Supported by FONDECYT grant 1080459.

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Auditory Stream Segregation in Gray Treefrogs, Hyla chrysoscelis

In humans, overlapping sounds can be segregated into separate "auditory streams" that can be selectively attended to based on differences in their acoustic properties. This capability for "auditory stream segregation" contributes to solving the so-called "cocktail party problem" of following one conversation in multi-talker social gatherings. The extent to which nonhuman animals rely on similar processes to hear communication signals in noisy social environments remains largely unknown. We tested the hypothesis that gray tree frogs are capable of auditory stream segregation based on frequency differences. Male gray tree frogs form dense choruses and produce pulsed advertisement calls (50 pulses/s) with spectral peaks at 1.3 and 2.6 kHz. Females are selective for conspecific pulse rates. In no-choice phonotaxis tests, we presented females with a ‘test stimulus’ in the presence of different ‘captor stimuli’. The test stimulus had the conspecific pulse rate and a single frequency peak at either 1.3 or 2.6 kHz. The captor stimuli consisted of continuous pulse trains that were interleaved with the test stimulus and had a constant frequency differing from that of the test stimulus between 3 and 15 semitones (in 3-semitone steps) across conditions. We predicted that at small frequency separations (DF), females would experience an integrated, "one-stream" percept with an unattractive pulse rate (100 pulses/s) at the time of the test stimulus. At larger DFs, we predicted females would experience separate auditory streams corresponding to an attractive call (50 pulses/s) and the captor stimulus. The proportions of females responding to the test stimulus were higher, and their response latencies lower, at larger compared to smaller DFs. These results confirm that female gray treefrogs were able to segregate interleaved sounds into separate auditory streams based on differences in frequency.

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P 215

Spatial release from masking in the anuran auditory system: Are two frequency channels better than one?

Acoustically communicating animals often face the problem of detecting and recognizing relevant signals in high levels of background noise. In humans, speech perception in the presence of a speech-like masker improves if the source of speech is spatially separated from the noise. This phenomenon, termed "spatial release from masking" (SRM), has also been demonstrated in gray treefrogs (Hyla chrysoscelis); both detection and recognition of species-specific mating calls improves when they originate from locations other than that of noise simulating a breeding chorus ("chorus-shaped noise"). In this study, we begin to explore the mechanisms underlying these observations. Because frogs have inner ears with two sensory papillae that are maximally sensitive to different frequency ranges, we tested the hypothesis that SRM could be mediated via acoustic inputs in either frequency range. In gray treefrogs, the amphibian papilla has a frequency range centered around 1300 Hz and the basilar papilla has a frequency range centered around 2600 Hz. Therefore, we presented subjects with one of three signals: a normal, bimodal call with spectral peaks at 1300 Hz and 2600 Hz, and two unimodal calls with a single frequency peak at either 1300 Hz or 2600 Hz. We broadcast signals in the presence of chorus-shaped noise in two treatment conditions: in the 'co-localized' condition, signal and masker were broadcast from the same speaker; in the 'separated' condition, signal and masker were spatially separated by 90°. Using an adaptive tracking procedure we measured signal recognition thresholds in each condition and determined the magnitude of SRM as the difference in threshold between the two conditions. We present results from these experiments and discuss implications for how SRM is mediated by inputs through the two sensory papillae of the amphibian inner ear.

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P 216

Call recognition by frogs in the presence of amplitude modulated “chorus-shaped” noise.

Despite the importance to animals of perceptually separating acoustic signals from background noise, we know relatively little about how they do it. In humans, temporal fluctuations in the level of background noise influence speech recognition thresholds and can both constrain and facilitate speech perception. Compared to listening in unmodulated noise, thresholds are reduced (i.e., "masking release" occurs) when maskers slowly fluctuate in amplitude. However, maskers modulated at rates more similar to the natural amplitude fluctuations in speech can elevate thresholds (i.e., "modulation masking" can occur). In this study of gray treefrogs (Hyla chrysoscelis), we asked whether amplitude modulations in the simulated background noise of a breeding chorus affect the ability of females to recognize male mating calls. Extensive field recordings indicate that the sounds of a gray treefrog chorus are reliably modulated both at slow rates (< ≈5 Hz) and at a faster rate characteristic of the pulsed structure of conspecific mating calls (≈45 Hz). In female phonotaxis experiments, we tested the hypothesis that thresholds for recognizing male mating signals are influenced by the presence of temporal fluctuations in chorus-like noise. Using rates of sinusoidal amplitude modulation (SAM) between 0.625 Hz and 80 Hz (octave steps), we found that slow rates of modulation (< 10 Hz) resulted in small improvement in thresholds (≈-2 dB masking release) compared to an unmodulated control. Rates of modulation above 10 Hz, however, resulted in elevated thresholds (≈+6-8 dB modulation masking). The highest thresholds were measured in noise modulated at 40Hz. These results indicate that modulation rates similar to those in signals (≈45 Hz) can actually impair vocal perception, suggesting that temporal fluctuations in chorus noise impose constraints on acoustic communication in natural social environments.

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552

P 217

Neuronal activations and behavioral preferences for father□fs song in female Bengalese finches

Male songs contain several song elements. The elements form several sequential patterns in Bengalese finches. We recorded behavioral preferences for their father□fs songs by an operant conditioning setup. In the first experiment, the father"s song and unfamiliar conspecific songs were presented as stimuli. Females showed preferences for the father□fs song (Wilcoxon signed rank test, $n = 6$, $p < 0.05$). The result suggested discrimination of the elements difference. In a second experiment, sequential differences were tested by the presentation of element-order-reversed songs (OREV) and the original father"s songs. If females could discriminate the song sequence, we predicted that females would prefer the normal father song than unnatural OREV. However, females did not exhibit any preferences for either type of song stimuli ($n = 7$, $p > 0.05$). To test the auditory processing of sequential differences, we measured the expression of the immediate early gene, ZENK as a marker of neuronal activity for songs. The song presentation induces ZENK in secondary auditory areas the caudomedial nidopallium (NCM) and the caudomedial mesopallium (CMM). However, the repeated presentation of a same song caused decrease of ZENK expression (Mello et al. 1995). We repeatedly presented female□fs father songs and after that, the experimental group was presented with sequentially shuffled father"s songs. If auditory areas process the difference of the sequence, we predicted that the shuffled song cause full ZENK expression. As a result, the ZENK expression was decreased as similar to the control group that presented only original father□fs song (ANOVA, $n = 21$, $p < 0.01$). Therefore, the auditory areas process differences of song elements rather than sequential information.

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P 218

Neural basis of cooperative behavior in the Plain-Tailed Wren (Thryothorus euophrys)

Male and female plain-tailed wrens cooperate in song production: males and females alternate syllables in a tightly coordinated duet song. As a first step towards understanding how sensorimotor systems in the brain mediate this behavior we made a detailed study of song production in these birds. We found that both males and females produce solitary songs that differ in the timing of the intervals between syllables to that of duet song. We also found that both males and females shift the frequencies of their song syllables during duets so that the frequencies are more similar to each other. To examine the representation of song in the brain we made extracellular recordings in HVC of urethane-anesthetized birds. Our preliminary neurophysiological data show that, as has been observed in several other species, HVC neurons are selective for the acoustic parameters of the duet song over other sounds. Further, HVC neurons appear to preferentially respond to playback of the autogenous syllables and not to those of the companion bird. Temporal combination sensitive neurons appear to encode autogenous syllables and not intervening syllables produced by the companion.

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P 219

Selective alteration of song production to study sensorimotor integration in zebra finches.

Songbirds has been widely studied as a model for vocal learning. As humans, they rely on hearing a tutor and own vocalizations during the learning process. Auditory feedback is also a key feature in humans and songbirds for maintaining adult vocalizations, as deafened individuals present gradually degradation of the vocal production. However, the mechanisms underlying the role of auditory feedback remain largely unknown. To allow for better experimental control of the auditory feedback a bird experiences during singing, we are developing a novel method for individual syllable manipulation. We have achieved fine control of the song production in zebra finches by modifying the subsyringeal pressure. A phonation pressure threshold can be defined as the minimum subsyringeal pressure required to initiate labial oscillation required for sound production. This threshold may be syllable dependent. We reduce the air sac pressure to subthreshold values by opening a flexible tube that is inserted in the air sac system. Dynamic control of the opening is achieved with a miniature valve (circa 10 ms opening time) that is attached at the end of the tube. The muting interval can be less than a syllable but extend to an entire motif, which is major unit of song production comprising multiple syllables. The advantage of this method is that for short duration disruptions, the motor gestures (defined as the activity of syringeal and respiratory muscles) are not directly compromised. It is important to notice that this method is reversible as the implant can be removed and the air sac sealed. Prior studies of the role of auditory feedback in song control in normal hearing birds have relied either on noise playback or delayed syllable playback. These have the disadvantage of superimposing external sounds onto feedback from the birdâ€™s singing. In the present experiment, we are studying the sensorimotor integration when no auditory input is received (muted syllable) and when the muted syllable is replaced with an acoustically modified syllable.

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635 **P 220**

RECONSTRUCTING THE HISTORY OF ACTIVITY OF AUDITORY NEURONS IN FEMALE SONGBIRDS

One common means to identify selective properties of sensory systems is through immediate early genes (IEGs). IEGs, however, can be disadvantageous because they require each individual be assessed under a single condition. One means to overcome this disadvantage is by examining multiple genes to reconstruct the activity history of an individual neuron. We use this approach, referred to as catFISH (cellular compartment analysis of temporal activity by fluorescence in situ hybridization) to identify auditory neurons activated by two auditory experiences in female songbirds. We examine Arc (activity-regulated cytoskeleton-associated protein), an IEG that appears within multiple cell compartments and is specifically linked to auditory processing in songbirds. We examine Arc mRNA in two auditory forebrain areas of female zebra finches following sequential exposure to two different songs or the same song twice. Females were exposed to conspecific followed by conspecific songs (same song twice) or conspecific songs followed by heterospecific, reverse conspecific, or noise (different songs). Arc was quantified in caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM). The cellular rate of reactivation indicates that distinct CMM cells respond to different songs, rather than the same cells responding to multiple songs. When females heard different songs there were a significantly greater number of cells expressing Arc in CMM in response to conspecific songs as compared to other song types, but not in NCM. These results indicate that CMM and NCM have distinct roles during song perception in female zebra finches: CMM cells function in song discrimination whereas NCM cells function in song recognition. These experiments provide insight into the auditory regions that give rise to sensory-biases involved in species recognition and discrimination

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P 221

Auditory responses in songbird telencephalon: tuning to familiar vocal communication signals

Songbirds provide a useful model for studying the neural code underlying the perception of acoustic communication signals. They rely on auditory processing of vocalizations for a number of social behaviors, such as pair-bonding. Vocalizations, songs as well as calls, are multiple cue signals that may convey information about the identity of the bird. An avian brain nucleus that is analogous to the mammalian secondary auditory cortex (the caudo-medial nidopallium or NCM) has recently emerged as part of the neural substrate for sensory representation of natural vocalizations. This led us to investigate whether, in the zebra finch, NCM neurons could contribute to the discrimination among vocalizations that convey information about the individual identity of the bird. We focussed on the long distance call. Females can indeed identify their mates on the basis of this call alone. We examined whether NCM neurons in females responded selectively to the mate call over familiar or unfamiliar calls. To this end, adult zebra finches (n=15 pairs) were paired for two months in the aviary while other females (n=7) remained unpaired. A few days prior the electrophysiological investigation, each pair was placed in a cage that allowed visual and acoustic contact with another pair (familiar pair). As a first step, single-units were performed in both paired and unpaired anesthetized females. Then, by using a telemetric device, we collected multi-unit responses in freely behaving birds. Results indicated that, in both anesthetized and awake paired females, neurons exhibited auditory responses of greater magnitude to either the mate or the familiar call than to the unfamiliar call, with no difference between the mate and the familiar call. In contrast, in unpaired females, no such differential responsiveness was observed. This suggests that response properties of NCM neurons may be tuned to familiar vocal communication signals.

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P 222

Functional MRI reveals a relationship between the strength of song learning and bird's own song selectivity in the auditory midbrain

Songbirds share with humans the capacity to learn their vocalizations. Both juvenile birds and human infants form memories of the adult vocalizations they hear and subsequently try to match their own vocalisations to these memories. For this reason, songbirds are a model of choice to study vocal learning and auditory memory processes. Behavioral studies indicate that songbirds perceive their own song differently from other conspecific songs. At the neural level, several brain regions involved in song production and learning have been identified as being selectively activated by the bird's own song (BOS) in the past years. In a previous functional Magnetic Resonance Imaging (fMRI) study, we have shown that BOS selectivity is present in the right ascending auditory pathway of adult zebra finches (Taeniopygia guttata) at the midbrain level (Poirier et al., 2009). However, because the birds we previously tested were coming from local suppliers, their rearing environment was not known. In a new fMRI experiment, we re-assessed BOS selectivity in adult birds that were housed singly with an adult male tutor during the sensitive period for vocal learning (dph (day post hatch) 43-100).This new study not only confirms the presence of BOS selective neural responses in the right auditory midbrain but also indicates that BOS selectivity is linked to the strength of song learning. More precisely, we found a significant positive correlation between the intensity of BOS selectivity in the right auditory midbrain and the degree of similarity between the BOS and the tutor song the experimental birds have copied. Because BOS selectivity might reflect BOS memory, and a good copy from the tutor song might reflect a good memory of the tutor song, this correlation suggests that BOS and tutor memories are tightly linked.

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P 223

Sound imaging system for visualizing spatio-temporal behavior of calling nocturnal animals

A sound imaging system for visualizing calling nocturnal animals" acoustic communication is presented. This system is applicable to small and gregarious animals such as frogs, crickets. Analyzing their calling behavior in the real field has been difficult with conventional approaches using (1) human"s ears and (2) data-loggers. The reasons are: (1) to distinguish many calling sounds is difficult for human"s ears, and, (2) data-loggers are inapplicable due to their size, because the size of target animals is about a few centimeters. To solve these problems, we have developed a novel sound imaging system which consists of newly developed sound imaging devices named "Firefly" and a commercial video camera [1]. Firefly is a 6-by-10-cm device equipped with a microphone and a LED (light emitting diode). The LED glitters when the microphone captures surrounding animals" calls. Our system works as follows: First, we deploy the Fireflies in the field and record the glittering patterns with a video camera. Second, we detect LED lights and extract glittering patterns of each Firefly for each frame. Then, we can visualize spatio-temporal calling behavior of nocturnal animals. The first experiment is conducted in a room to verify the performance of our sound imaging system using two Japanese tree frogs [2] (Hyla-japonica) in cages individually. The result confirms that our system visualizes the calling behavior. The second experiment is a field feasibility test to investigate frogs" calling behavior in the field. We have observed one phenomenon that two frogs call in anti-phase synchronization in the field with about 30cm spatial and 30Hz temporal resolution. This result demonstrates that our system can visualize not only the location of the calling animal, but also its temporal characteristics. [1]T. Mizumoto, I. Aihara et al."Sound Imaging of Calling Nocturnal Animals", in preparation. [2]I. Aihara, Phys. Rev. E 80, 011918 (2009).

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685 **P 224**

Measuring mouse vocal and social behaviour

Vocal behavior is the product of a complex interaction between the neural circuits that underlie auditory perception and those that underlie vocal production. In addition, vocal signals are often elicited by, or modulated by, social context. For example, many rodents use ultrasonic vocalizations during social encounters. The ease with which vocalizations are recorded, and their salience for the animal, makes vocalizations a favorite target of neuroethologists. The power of the neuroethological approach is amplified in the house mouse (Mus musculus) because of the added possibility of sophisticated genetic manipulation techniques and the practical consideration of the vast number of different mouse strains readily available. Mouse vocal behavior therefore represents a potentially powerful model system in which to address the neural basis of complex social and motor behaviors. To study the full vocal and social behavioral repertoire of the mouse you need cages in which multiple social contexts associated with vocalizations occur, the ability to track multiple individual mice over socially relevant timescales, the ability to record vocalizations continuously during social interactions, and a method for quantifying vocal structure, to compare vocalizations across different social contexts. We have developed a combination of video- and RFID-based methods to track the position and identity of group-housed mice in a 2’x2’ enclosure over multiple days. Mice are bleach marked to allow unambiguous identification. Four ultrasonic microphones record vocalizations continuously during social interactions. We use hidden Markov models (HMMs) to describe the structure of ultrasonic mouse vocalizations across social contexts.

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P 225

Developmental changes in CBA/CaJ mouse vocalizations: an analysis of song structure.

Mice, both pup and adult, produce a variety of social communication calls. Our study examined developmental changes in these calls between post-natal day 4 (p4) and adulthood. We investigated if there are developmental differences in the types of calls produced, their spectro-temporal characteristics, and the pattern of occurrence. Recordings were obtained and analyzed with an Avisoft system using a sampling rate of 500 kHz. CBA/CaJ pup calls were recorded for 5 minutes of isolation from 15 pups at ages p4 (n= 3145), p6 (n=4329), p8 (n=6306), p10 (n=4560) and p12 (n=3082). A total of 6963 adult calls were recorded in various social settings; male-male, male-female, female-female, and male with the bedding from a female"s home cage. We classified 11 call types based on spectro-temporal characteristics, all but one were produced by both pups and adults. The Chevron call became increasingly common with age, whilst the 1 frequency stepped call become steadily less common. Most calls became shorter with age; adult calls were 40 to 70% shorter than calls of young pups. Only the upward FM call increased in duration with age (by 11 ms). The dominant frequency of different call types reduced by 12-20 kHz between age p4 and adulthood. As pups aged, the proportion of calls with nonlinear components increased steadily from 0.3 % at p4 to 23.5% at p12, however, nonlinear calls were rare from adults (1.5%). The temporal characteristics of call bouts changed with age, older pups and adults had shorter intervals between calls and fewer calls within bouts. Although the majority of call types were produced by both pups and adults, they had different probabilities of occurrence at different ages. The vocal repertoire became more diverse and less repetitious over age. The complexity of sequences of call types within bouts increased with age. These differences in probability, duration, dominant frequency and bout structure may be used by other mice to determine the age of the caller.

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Cadherin-6 knockout mouse shows defects in ultrasonic vocalization

Mice use ultrasonic vocal signals for intraspecific communication. Mouse pups emit an ultrasonic isolation call when they are separated from their mother and littermates. Recently, it has been described that adult male mice emit ultrasonic vocalizations when they encounter estrus female mice, and that the vocalizations have the characteristics of the song (Holy and Guo, Pros Biology. 2005). In the previous study using the Bengalese finch, a species of songbirds, we found that perturbation of cadherin expression in vocal nucleus with lentiviral vectors showed severe defects in song development (Matsunaga & Okanoya, Soc. Neurosci. Abs. 2008), suggesting that cadherins play essential roles in vocalization of songbirds. In this study, we used cadherin-6 knockout mice to investigate the function of cadherins in mice vocalization. We recorded the ultrasonic vocalization in two different social contexts: pup´s isolation context and adult courtship context. In both contexts, the pitch of calls in cadherin-6 knockout mice was changed compared to wild types. In contrast, other components of vocalization like number of calls and latency of first call were not affected. Additionally the deficit in cadherin-6 knockout was vocalization-specific, and there are no defects in other general behavioral abilities. These results indicate that function of cadherins may be partly conserved in mice vocal behavior as songbirds.

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Electrosensory Systems

655 **P 227**

Electrocommunication modulations called chirps signal aggressive intent in a species of weakly electric fish, Apteronotus leptorhynchus

The brown ghost knifefish, Apteronotus leptorhynchus, is a Gymnotiform species of weakly electric fish that use electric signals to modulate conspecific behaviour during social interactions. Electric fish produce an electric signal and detect perturbations in its electric field and other electric signals in its environment including those generated by predators, prey and conspecifics. During aggressive interactions, conspecifics produce stereotyped frequency modulations of the electric signal termed chirps. In staged free-swimming dyadic interactions, male A. leptorhynchus tend to produce chirps antiphonally between bouts of aggression. Here we use an interactive chirp playback paradigm to characterize the chirp and aggressive behaviours produced in response to a simulated intruder, and provide results that support the hypothesis that chirps signal aggressive intent in male A. leptorhynchus. Fish that chirp in response to a simulated intruder respond with significantly more aggression than do fish that do not chirp towards a comparable intruder. Additionally, we found pronounced inter-individual differences in chirp and aggression responses to the playback intruder. We asked whether individual differences in playback performance predict aggression and boldness scores across multiple behavioural tasks (in exploratory, novel object and feeding scenarios). We found significant correlations in aggression and boldness across different contextual tests. For the first time, we provide evidence for aggressive behavioural syndromes in a species of weakly electric fish, and suggest that individual variations in aggression are predictive of chirping behaviour during conspecific interactions in A. leptorhynchus.

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P 228

Encoding of communication signals at different contexts in a wave-type electric fish

Weakly electric fish use their electric organ discharge (EOD) for electrolocation as well as for communication. One class of communication signals are so-called chirps, transient increases in the frequency of their otherwise very stable quasi-sinusoidal EOD. In agonistic contexts, Apteronotus leptorhynchus emit type II chirps with a small frequency excursion and a short duration. When two fish interact, both their EODs superimpose to a beat, an amplitude modulation of the frequency difference between the two EODs. Chirps cause an amplitude and a phase modulation of this beat, which is encoded by the electroreceptor (p-unit) population. As has been shown, the response of p-units to beats and chirps depends strongly on the beat frequency. The EOD frequency of each fish depends on its gender, size and social status. Hence, different beats reflect different social encounters. At intermediate beat frequencies around 60Hz the p-unit population activity is synchronized by the beat. At slow and fast beats, on the other hand, the receptors fire asynchronously. Until recently, small chirps were thought to be relevant only at low beat frequencies up to 30Hz. In this regime they are emitted most frequently and encoded by a strong increase in the population response. A recent study has shown, however, that behavioral responses to type II chirps such as attack rates, increase with beat frequency. Until now, it is not known how chirps are encoded at these higher frequencies. We here analyze results from single-cell-recordings of p-units in response to chirps at different beats. At beat frequencies above about 80Hz, small chirps reduce the population response. This completes the picture as it is expected from the population response to different beats: small chirps synchronize cells at low beats, while they desynchronize them at high beats. This suggests different encoding strategies in the electroreceptive system depending on the context in which it receives communication signals.

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P 229

Modulation of aggressive behavior by previous social experience in an electric fish

Animals adjust their behavior by integrating environmental cues and information gathered from social interaction. During aggressive encounters, individuals evaluate costs and benefits of being involved in an escalated conflict. Depending on previous social experience, animals might adopt different social strategies according to their estimated probability of future success (winner-loser effect). It has been demonstrated in vertebrates that these behavioral modulations depend on steroid hormonal variations. We used the weakly electric fish, Gymnotus omarorum (highly aggressive, territorial, seasonal breeder), as the model system for the study of agonistic behavior and its modulation. Aggressive interactions in this species involve specific and stereotyped locomotor and electric displays. To establish the effects of previous contest outcome on future contests across seasons, we set up a first dyadic interaction involving naï¿½ve individuals that therefore acquired loser or winner experience. We then tested these individuals against pair-sized opponents at two different time scales -1 hour and 1 week- to establish the decay time of these effects. Previous winners significantly increased their winning probability against pair-sized rivals across seasons, except for females that did not show winner effect during breeding. Previous winners also exhibited higher levels of testosterone than naï¿½ve individuals. Secondly, contests tended to be escalated and lasted longer than those observed in control groups. Individuals with loser experience lost significantly more contests and loser effect was observed across seasons in both males and females, although females did not show loser effect 1 week after. Striking changes in behavior were also documented: contest duration was extremely reduced and submissive electric signals changed in quality and increased both in emission rate and duration. This is the first report that demonstrates that experience is associated to modulation of electric behavior. Partially supported by ANII FCE2007_569 and PEDECIBA

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Behavioral and cellular bases of vasotocin modulation of agonistic behavior in two species of weakly electric fish.

Social behavior in vertebrates is controlled by a complex and conserved brain network. Inter and intraspecific modulations of behavior are correlated with distinctively distributed patterns of activity in this network, which depend on neuroendocrine messengers that integrate extrinsic and intrinsic cues. The neuropeptide arginine- vasotocin (AVT) and its mammalian homologue, vasopressin, are key integrators underlying interspecific, sexual, individual, and social context differences in behavior across vertebrate taxa. Comparative studies of AVT actions between related species with different social structure have been useful for the understanding of the neural bases of behavior. We used two species of weakly electric fish with different social strategies: Gymnotus omarorum, territorial and highly aggressive, and Brachyhypopomus gauderio, gregarious and only aggressive during the breeding season. We focused on electric organ discharge (EOD) rate modulations, which is one of the components of social behavior in electric fish. AVT produces an immediate and sustained increase of the EOD basal rate of B. gauderio, and has no effect on the basal EOD rate of G. omarorum. These results suggest that AVT acts directly on the pacemaker nucleus (PN) in B. gauderio but not in G. omarorum. Anatomical differences in AVT neurons and projections to the PN were explored in both species. Vasotocin-positive neurons somata in the POA of both species were found. Only B. gauderio showed vasotocin- positive fibers projecting to the PN. Submissive electric displays (chirps and interruptions) are produced by subordinate fish during agonistic behavior in both species. These social electric signals depend on pre-pacemaker inputs to the PN. The administration of AVT to putative subordinates in dyadic agonistic encounters allowed us to discriminate the action site of AVT in the modulation of EOD rate in this species. Partially supported by ANII FCE2007_569 and PEDECIBA

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P 231

Neural basis for seasonal social signals: a study of glutamate receptors in a pacemaker nucleus

The cyclic enrichment of behavioral repertoires is a common event in seasonal breeders. The teleost Brachyhypopomus gauderio produces electric organ discharge (EOD) rate modulations only during the breeding season as part of its courtship behavior, in a dialogue of male "chirps" and female EOD interruptions. The EOD rate is commanded by a medullary pacemaker nucleus (PN) composed of pacemaker neurons and projecting relay neurons. The relay population receives pre-pacemaker inputs to generate chirps, reportedly mediated by AMPA receptors, and EOD interruptions, mediated by NMDA receptors. Recent reports have shown that seasonal plasticity and sexual differences in the production of chirps lay, at least in part, in the PN. We have focused on the study of glutamate receptors which may underlie the seasonal cycling of chirp generating capacity in the male PN. We used breeding and non breeding male and female adults to perform in situ hybridization to identify the presence of mRNA encoding for different subunits of the NMDA receptor: NMDAR1 and NMDAR2B. Both breeding males and females showed similar staining indicating NR1 and 2B in both pacemaker and relay neurons. Non breeding animals, on the other hand, exhibited a stronger 2B presence in relay cells in comparison to their own pacemaker cells and to those of breeding adults. In other vertebrates, NDMDA2B has been seen to increase in inverse correlation to androgen levels. Given the recent reports of seasonal cycling of androgen receptors in the male PN of this species, the seasonal differences in NMDA2B mRNA suggest this subunit may be involved in the cycling of social signal production. We are also interested in investigating AMPA receptor mRNA by in situ hybridization. For this, we have partially cloned the GluR2B gene for this species, obtaining a 903 bp sequence which has over a 85% match with other teleost fish. The analysis of seasonal and sexual differences in AMPA subunit mRNA presence is currently underway. Financed by PDT 043, IBRO, PDT 55/29, IDRC

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P 232

Interspecific differences in agonistic behavior and its serotonergic modulation

Agonistic behavior is universal across evolution and its neural control mechanisms are highly conserved. Although high levels of 5HT are usually correlated to subordination among vertebrates, there are few reports that link serotonergic modulation with interspecific or phenotypic differences in aggression. We propose a comparative analysis to understand the neuroendocrine bases of aggression and its serotonergic modulation in two species of electric fish with different social structure. Brachyhypopomus gauderio is gregarious, only aggressive between males during breeding season whereas Gymnotus omarorum is territorial and highly aggressive regardless sex and season. We first described and quantified agonistic behavior (locomotor and electric displays). The variables that influence conflict resolution are different between species being weight the only variable that matters in G. omarorum all year round, whereas in B. gauderio length is also important. Quality of agonistic behavior was also different between species: dynamics were faster in G. omarorum, aggression levels (attacks and bites) and submission levels (latency to the first off and total time off) were higher in G. omarorum than in B. gauderio. Tisular levels of 5HT were quantified using HPLC in different regions of the CNS. We found significant dominant-subordinate differences in both species with higher levels in subordinates. In the interspecific comparison, dominants and subordinates males of B. gauderio showed higher telencephalic levels of 5HT than G. omarorum. Finally, the role of 5HT1AR upon different components of agonistic behavior in both species was explored using 8OH-DPAT and WAY100635. As expected, serotonergic modulation had clear effects in G. omarorum but unapparent in B. gauderio. These data reinforce the value of our experimental model for the understanding of differences in the neural control of adaptive aggression and its transition to violence. Supported by ANII FCE2007_569, PEDECIBA

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P 233

Molecular characterization and localization of the serotonin 1A receptor in the brain of the male gymnotiform electric fish Brachyhypopomus gauderio.

Serotonin, a neurotransmitter with a wide range of effects in the central nervous system, plays a major role in regulating aggressive behavior. The relationship between serotonergic activity and regulation of aggressive behavior depends on the type of serotonin receptor, the quantity and location of the receptors, and the intracellular signaling pathway activated by the receptor. The serotonin 1A receptor, 5HT1AR, is a key player in the regulation of aggressive behavior. The gymnotiform electric fish Brachyhypopomus gauderio generates dual-function electric signals for electrolocation and communication. Social interactions, the serotonergic system, steroid hormones, and melanocortins regulate the electric signal waveform. Pharmacological activation of the 5HT1AR reduces the magnitude and duration of the electric waveform in male B. gauderio. To further characterize the role of the serotonergic system in the regulation of gymnotiform male electric signals, we isolated RNA, cloned, and sequenced the 5HT1AR receptor from the brains of B. gauderio males. Our sequence analysis suggests that B. gauderio’s 5HT1AR is homologous to and phylogenetically more-closely related to the b/â isoform found in other teleost fish. We confirmed the presence of previously reported and identified putative novel phosphorylation sites in the 5HT1AR of B. gauderio and other teleost fish. Using the zebrafish genome as a template, we also identified a putative glucocorticosteroid response element in the promoter region of the teleost 5HT1AR b/â gene. In situ hybridization of brain sections labels 5HT1AR mRNA in the ventral and dorsal subdivisions of the telencephalon, areas believed to function similarly to the mammalian amygdala, the raphe nuclei, several hypothalamic nuclei, and electrosensory-motor nuclei. Taken together, these findings highlight how electric signal plasticity may be under a bidirectional regulatory loop between the hypothalamic-pituitary-adrenal axis and 5HT1AR in gymnotiform fish.

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P 234

Black ghost knifefish electrically jam simulated rivals during aggressive interactions

Previous studies have demonstrated that one weakly electric fish, the brown ghost knifefish (Apteronotus leptorhynchus) uses electrical frequency jamming as an aggressive behavior during social interactions with conspecifics of the same sex. Brown ghosts also jam simulated rivals in the form of a playback signal mimicking a conspecific. Our current study demonstrates that highly aggressive black ghost knifefish (Apteronotus albifrons) also exhibit frequency jamming in same-sex social interactions. Black ghosts presented with a stimulus frequency mimicking a conspecific raised their electric organ discharge frequency to jam the stimulus signal while directing attacks at the playback electrodes. Preliminary results from a second experiment suggest that exposure to the selective serotonin reuptake inhibitor fluoxetine alters jamming behavior, indicating that serotonergic pathways may be involved in jamming.

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The Effects of Fluoxetine on Physical Aggression in a Weakly Electric Knife Fish, Apteronotus albifrons

Serotonin is a neuromodulator that can affect various behaviors across vertebrate species. Regulating the amount of serotonin present within the neural synapse can dictate aspects of aggression and reproductive behavior. Fluoxetine is a serotonin reuptake inhibitor that increases the amount of serotonergic activity within the brain. Few studies have been able to clearly define the relationship between physical aggression and serotonin because aggression is an extremely complex and dynamic behavior that can manifest in various fashions. This study demonstrates the correlation between fluoxetine-mediated serotonin levels and physical aggression in weakly-electric black ghost knife fish (Apteronotus albifrons). This study also suggests a correlation between dose and response implying that physical aggression is mediated through a neural serotonin pathway.

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The Effect of Bisphenol A on the Electric Organ Discharge of Weakly Electric Fish

Bisphenol A is a known endocrine disrupting chemical (EDC) that is incorporated into many modern plastics such as baby bottles, water bottles, and reusable food storage containers, as well as the linings of many aluminum food cans. EDC pollutants interfere with the metabolism, transportation, and binding of hormones and may have harmful effects on the hormonal systems of fish and other vertebrates leading to problems in social communication, gender recognition, mating success, and aggression recognition. BPA has been shown to exhibit endocrine disrupting behavior in exposed fish by disrupting sexual differentiation and male-to female sex reversal, malformation of eggs and fry, reduced egg production rates, and reduced egg hatching rates. This chemical's estrogenic method of action and its persistence in the environment pose a potential risk to fish and other aquatic life, and we predicted that it would cause a measurable effect on the electric organ discharge (EOD) of weakly electric fish. We exposed Apteronotus albifrons, black ghost knifefish, to different environmentally relevant concentrations of BPA. Preliminary data indicate that exposing black ghost knifefish to low concentrations of BPA feminizes the EOD frequency, thus providing a behavioral biomonitoring model system for the testing of similar suspected EDC pollutants.

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P 237

Short-term physiological and behavioural measures support low metabolic cost of electric signal generation by wave-type weakly electric fish

Fundamental trade-offs in energy allocation of an organism are often difficult to identify, but may become apparent under extreme conditions. Hypoxia (low oxygen) can operate as a significant environmental stressor, particularly for water-breathing organisms given the high costs of oxygen acquisition. Gymnotiform weakly electric fishes generate electric organ discharges (EODs) and sense perturbations of the resulting electric field for purposes of orientation, prey detection, and communication. Some species produce oscillatory ("wave-type") EODs at very high frequencies (up to 2 kHz) that have been proposed to be energetically expensive. If high-frequency EODs are expensive, then fish may modulate their EOD frequency in response to hypoxic stress or they may compensate for costs of signaling through other adaptations that maximize oxygen uptake efficiency. These fishes generally occur in high dissolved oxygen (DO) waters, although some species are known to inhabit low-DO waters. We asked whether there is evidence for an energetic cost of signaling in two species that differ in their hypoxia tolerance. We recorded the EOD in conjunction with oxygen consumption rates, critical oxygen tension, and aquatic surface respiration (ASR) thresholds in Apteronotus leptorhynchus (high DO) and Eigenmannia virescens, a species more typically found in low-DO waters. E. virescens had a lower mean ASR threshold and critical oxygen tension compared to A. leptorhynchus, consistent with field distributions. Results indicated a weak positive relationship between EOD frequency and DO in both species. There was no relationship between RMR and EOD. These results do not support a high metabolic cost of electric signalling on short time scales. We are currently exploring the long-term impacts of hypoxia on characteristics of the EOD.

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The Metabolic Cost of Electric Signaling in Wave-Type Weakly Electric Fish

Wave-type weakly electric fish emit a highly regular electric discharge that is produced by a specialized electric organ in the tail. This quasi-sinusoidal electric organ discharge (EOD) creates an electric field in the water surrounding the fish that is used for navigation, prey detection, and communication. Recent research suggested that pulse-type electric signaling constitutes a significant portion of the fish's energy budget (Salazar and Stoddard, 2008, JEB, 211, 1012-1020), yet the metabolic cost of wave-type electric signaling has not been studied extensively. In the current study, metabolic rate was manipulated via exposure to hypoxia and exercise, and the effects of these manipulations on the EOD were examined. In addition, the jamming avoidance response (JAR) and long term frequency elevation (LTFE) (Oestreich and Zakon, 2005, J Comp Physiol A, 191, 845-856) were used to compare metabolic rate for different EOD frequencies in an individual fish. Both Apteronotus leptorhynchus and Eigenmannia virescens maintained a nearly constant EOD frequency despite a significant reduction in metabolic rate and reduced oxygen availability during hypoxic exposure. In A. leptorhynchus, oxygen consumption increased with swimming speed, but EOD frequency increased significantly with swimming speed, reaching a peak frequency increase of approximately 5% (up to 50 Hz); the effect of small increases in temperature (+0.5ºc) during swimming trials does not appear to account for the observed increases in frequency. Preliminary examination of EOD frequency elevation during JAR and LTFE revealed no significant change in oxygen consumption; however, a trend for increased oxygen consumption during JAR was observed in some individuals. Together, these data indicate that electric signaling in wave-type weakly electric fish is a not a major contributor to whole-animal energetic cost.

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P 239

Reproductive output of *Brachyhypopomus gauderio* decreases throughout the breeding season.

Life-history theory predicts a trade-off between current reproduction and, future reproduction and survival. When future reproductive opportunities are high, animals are expected to invest less in current reproduction. Conversely, animals are expected to boost reproductive investment at the end of their lives before future reproductive options disappear altogether. In Uruguay, the gymnotiform electric fish *B. gauderio* breeds by fractional spawning throughout the Austral summer, after which adults disappear from the population. The electric organ discharge (EOD) of *B. gauderio* is energy intensive, its cost varying with pulse duration, a sexually dimorphic signal character regulated by androgens. We questioned whether *B. gauderio* would increase its reproductive effort at the end of the season as predicted from its life history or at the beginning, when their offspring will have more time to grow before the winter. Thus, we conducted a field study to understand how social signals, steroid hormones, and reproductive effort vary in accordance with conflicting predictions of life history theory. In both sexes, serum androgen levels (testosterone and 11-ketotestosterone), EOD duration, and gonadosomatic index (GSI) were higher earlier in the breeding season than later, with no late season rise. Both androgens correlated with EOD duration and GSI in both sexes, with the exception that 11-KT did not correlate with GSI in females. Cortisol showed no seasonal pattern or correlation with other traits. Our data are consistent with two explanations for changes in seasonal effort: 1) individuals that expend the highest effort early in the breeding season experience high mortality and are replaced by lower investing, later maturing individuals, or 2) individuals invest early and reduce their reproductive output across the season.

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P 240

Cellular mechanisms of developmental and sex differences in plasticity of a social communication signal

In response to changes in the social and environment, some gymnotiform electric fish species rapidly modify their electric signal waveforms by altering the action potential (AP) waveforms of their electrocytes, the excitable cells that produce the Electric Organ Discharge (EOD). The real-time relationship between behavior and changes in cellular excitability are therefore particularly direct in this system. In the gymnotiform Brachyhypopomus gauderio, EOD amplitude and waveform are regulated by the coordinated timing and shaping of two distinct APs generated from two opposing excitable membrane faces in each electrocyte. The two membrane faces must fire in the proper order within 100 microseconds of each other and the second AP must be significantly broader than the first. We have shown previously that mature males increase EOD amplitude and duration when melanocortin peptide hormones act directly on electrocytes to selectively broaden the second AP and increase the delay between the two APs by approximately 25 microseconds. Here we report that melanocortins also increase EOD amplitude and duration in juveniles and mature females, but by different cellular mechanisms. Females selectively broaden only the second AP as males do, but increase amplitude of both APs with no change in delay between them. Juvenile fish broaden both APs and increase the delay between the APs. These results suggest that the particular cellular mechanisms of EOD plasticity are shaped during development, presumably by sex steroids, becoming sexually dimorphic at maturity. This system therefore offers a unique window for investigating how peptide and steroid hormones interact to shape an ongoing behavior by regulating the intrinsic plasticity of excitable cells in the microsecond domain, and modulating different AP thresholds and waveforms in different cellular compartments.

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 P 241

Evidence of differential gene transcription between electric organs and skeletal muscle in the mormyrid *Brienomyrus brachyistius*

Electric organs (EOs) have evolved independently in six different lineages of fish, and derive during development from skeletal muscle (SM). In South American electric fish (Gymnotiformes), the transition between SM and EO is associated with the down-regulation of sarcomeric proteins, and is facilitated by motorneurons that innervate the EO. African electric fish (Mormyriformes), unlike gymnotids, retain sarcomeric proteins in their EOs, and the SM-EO transition occurs in the absence of innervation. These prominent differences in EO development motivated our characterization of transcriptional differences between SM and EO in mormyrids.

We used Suppressive Subtractive Hybridization (SSH) to compare transcribed mRNAs. Forward and reverse SSH was performed on pooled SM and EO samples from five individual *Brienomyrus brachyistius*. We randomly selected 143 EO clones and 38 SM clones for sequencing (average length = 647bp), and attempted to identify each by BLAST searching NCBI databases. After confirming differential expression using RT-PCR, we utilized immunohistochemistry and western blotting to confirm translation into protein.

We detected 90 uniquely expressed transcripts, and identified 25 transcripts homologous to known genes. In EO these mRNAs encode proteins (1) responsible for the transport of ions (e.g. Na+/K+ pumps, Ca++ pumps), (2) that bind calcium (i.e. S100, parvalbumin, and troponin) (3) sarcomeric proteins (i.e. tropomyosin, myosin heavy chain), or (4) transcription factors (e.g. Myocyte Enhancing Factor 2A, *MEF2a*). Each of the above-described mRNAs is translated into protein; reverse translated sequences from EO were compared to SM sequences and to homologues in other vertebrates. Several mRNAs in EO encoded altered amino acid sequences. Our results suggest alternative functional roles of these proteins in EO, and that EO development in mormyrids may not be due to post-transcriptional regulation, as suggested in gymnotids.

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Comparison of active and passive electrosensory system in wave-type electric fish

The role of noise in neural information processing is still a controversial topic. The notion that intrinsic noise inevitably degrades information transmission still dominates the debate. However, in a population of spiking neurons noise can reduce redundancy and/or control the level of synchrony in response to certain stimulus features. Target neurons may selectively read out synchronous spikes only (coincidence detector) or unspecifically integrate over all input spikes, thus extract different aspects of the signal that stimulated the population of input neurons. In this view the amount of noise in a neural system might be optimally tuned to achieve the required signal processing properties. Weakly electric fish are an ideal model system for tackling this question experimentally. They have two parallel electrosensory systems that share a similar architecture and process similar stimuli but exhibit different response characteristics/variability: the active electrosensory system receiving input from tuberous receptors and the passive one receiving input from ampullary receptors. Interestingly, the baseline discharge of P-units, the majority of the tuberous receptors, show a much larger variability of their interspike intervals than the one of the ampullary receptors. Thus, the target neurons of the respective systems receive input from populations of receptor neurons that discharge more (ampullary receptors) or less (tuberous receptors) regular. These two systems are thus very well suited to allow for a comparative study on the role of noise. We here present, as a first step, recordings from the receptor neurons of both systems. We compare baseline firing rates, interspike interval distributions, and interspike interval correlations in order to better quantify the variability of the spiking responses (the noise). By means of sinusoidal and random amplitude modulations we quantify the tuning of both receptor types to dynamical stimuli.

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The gross anatomy and neural innervation of the Schnauzenorgan in the weakly electric fish, Gnathonemus petersii

During active electrolocation the weakly electric fish Gnathonemus petersii is using its movable chin appendix, the Schnauzenorgan (SO), like a probe to scan the ground or the surface of objects. The SO is a specialized anatomical structure that is moved in a characteristic manner. TheSO is hypophesized to constitute an electrosensory fovea with a special role in detection of nearby objects. In this study, we investigated the inner morphology of the SO enabling its complex movements, as well as the motor and electrosensory components of its neural innervation. For anatomical investigations, Schnauzenorgans were cut and their inner structure was reconstructed. The inner gross anatomy of the SO consists of a rod-like cartilaginous structure that extends to almost the tip of the SO. Along the cartilage, the muscles interdigitate and extend from dorsal to ventral within the SO. Several dorsal muscles fibers expand over two thirds of the length of the cartilage and therefore can cause an active upward movement of the tip of the SO. Most of the muscles are relatively short and spread over only a small and distinct part of the SO, allowing precise movements. The muscles are innervated by the Vth cranial nerve. The corresponding motor neurons are located in the trigeminal motor nucleus (nVm). Their axons leave the brain ventro-medial to the caudal part of the preeminential nucleus and run with the motor root of V. They enter the SO medially and run along the cartilage and between the muscle fibers. In addition to the motor fibers, the SO is innervated by electrosensory nerve fibers of the anterior lateral line nerve (NLLa). The NLLa enters the SO as a thick nerve bundle closely under the skin and splits near the base of the SO into several thinner branches. In addition to the NLLa, other sensory nerve fibers running in the Vth cranial nerve are also found in the SO.

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P 244

Detection of objects in complex 3-dimensional scenes by electrolocating Gnathonemus petersii

The weakly electric fish G. petersii generates electrical signals and builds up an electric field. The animals perceive their own electric emissions with epidermal electroreceptor organs. During active electrolocation, objects are distinguished by analysing the electric images, which they project onto the skin of the fish. In my project, I determined if G. petersii can discriminate objects of different shapes and sizes at different distances and in front of complex backgrounds. Fishes were trained in a food-rewarded two-alternative forced-choice procedure to choose one of two objects by swimming through one of two gates in a partition in the tank. My results show that G. petersii is able to discriminate between metal objects of different sizes and shapes up to a distance of about 4 cm. Subsequently I measured whether the presence of different backgrounds directly behind the objects (non-moving metal-, plastic- or water plant-, and moving plastic- or water plant backgrounds) can interfere with object discrimination. While static metal background effected object discrimination negatively, discrimination performance was significantly better when the objects were placed in front of a moving plastic or water plant background. This indicates that movements in the environment play an important role for object identification. In the future, I am planning to measure the electric images of the objects to define the parameters on the basis of which G. petersii discriminates between them.

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P 245

Representation of accurate timing by time-locked neurons in the midbrain of a pulse type electric fish, Brachyhypopomus gauderio

Pulse-type gymnotiform electric fishes possess a medial large nucleus in the dorsal half of the torus semicircularis in the midbrain. This mesencephalic magnocellular nucleus (MMN) does not exist in wave-type gymnotiform electric fishes but contains neurons morphologically similar to the time coding neurons in the lamina VI of the torus semicircularis in wave-type gymnotiform fishes. We have recorded intracellularly from these neurons and characterized their temporal properties. All neurons recorded in the MMN responded with a single action potential to an electrosensory stimulus pulse (a single sinusoid, 1 millisecond duration) and showed no spontaneous activity at all between stimulus pulses or to the absence of stimulus. Presentation of a sinusoidal or square pulse with various durations revealed that inward current into the fish body is associated with the generation of action potential in all neurons in the MMN. Latency measured from inward zerocrossing of stimulus to the initiation on action potential was 0.7 – 1.4 milliseconds. Standard deviations of latency (jitter) of continuously generated action potentials (20 Hz stimulation for 10 seconds, n = 200) ranged from 0.6 to 1.4 microseconds. These time-locked neurons responded with an action potential to every sinusoidal pulse presented at high frequencies (300 to 450 Hz). How is the accurate presentation of stimulus pulse timing by these neurons utilized for behaviors? We measured novelty responses – a transient increase of electric organ discharge frequency using the ⍰ephase chamber⍰f in which fish⍰fs head and trunk skin areas were electrically isolated. Pulse time shifting within and across skin areas demonstrated that differential times between the moments of inward current at different skin surface drives this behavior. The result suggests a neuronal mechanism that detects action potential time differences between MMN neurons with high accuracy.

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P 246

Histological studies for time-coding pathway in pulse-type Gymnotiform fish, Brachyhypopomus gauderio

Pulse-type weakly electric fish, Brachyhypopomus gauderio, shows behavioral sensitivity to microsecond time differences between electroreceptors in different body areas. To understand anatomical basis for the high temporal sensitivity we studied the fish"s time-coding pathway☐\ the electrosensory afferents, electrosensory lateral line lobe (ELL) and magnocellular mesencephalic nucleus (MMN) by biocytin histochemistry combined with electron microscopy (EM). We labeled afferent fibers by placing a piece of biocytin crystal on a cut surface of a nerve. The labeled afferents project to deep fiber layer in the ELL. Some terminals of the labeled afferents are found to be calyx-shape and terminated in the layer where adendritic ☐epear-shaped cells☐f (or ☐espherical cells☐f, soma size ~20μm) are aligned. The calyx terminals make synaptic contact with a soma of the cell. By placing a piece of biocytin crystal into the MMN, the pear-shaped cells are retrogradely labeled in the ELL whose axon goes through the mesencephalic torus semicircularis. To label neurons projecting to the MMN, we iontophoretically injected biocytin into commissure. The labeled terminals in the MMN, possibly of the pear-shaped cells, are glomerular with fine terminals. In the MMN we found two cell types by conventional EM: small cell (soma size ~10μm) and large cell (soma size ~30μm). The small cell soma receives a calyx terminal through gap junctions. The large cell soma is partially covered by myelination and relatively large unidentified terminals contact other areas of the soma. The global connections from the sensory afferents to the MMN correspond to the ☐efast electrosensory pathway☐f described in other pulse-type gymnotiform fish, Gymnotus, and these morphological characteristics suggest specialization to temporal coding in the microsecond range.

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P 247

Temporal Selectivity in Midbrain Electrosensory Neurons Identified by Modal Variation in Active Sensing

Mormyrid weakly electric fish actively sense their surroundings by continuously emitting discrete pulses of electricity separated by varying intervals of silence. The temporal pattern of this pulsing behavior is related to context. While resting in the absence of an overt stimulus, baseline interpulse intervals (IPIs) mostly range 200 – 450 ms and sequential variation is relatively high. Spontaneously, or following the presentation of a novel stimulus, IPIs transiently shorten during the performance of an electromotor "burst" display. We made intracellular whole-cell recordings in vivo from neurons in the lateral nucleus of the torus semicircularis while the fish's dynamic pulsing behavior modified the temporal pattern of stimulation. Stimulation was designed to simulate the spatial patterns of amplitude modulation that occur during the electrolocation of a resistive object. We discovered that toral neurons selectively respond to stimulation during a particular mode of electromotor activity. Two types of temporally selective neurons were discovered: (1) baseline-selective neurons that displayed significantly higher PSP amplitude and spike count per EOD during baseline electromotor activity, and (2) burst-selective neurons that displayed significantly higher PSP amplitude and spike count per EOD during electromotor burst displays. Interval-dependent changes in the strength of excitation and inhibition contributed to their selectivity.

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P 248

Neural heterogeneity and efficient population codes for communication signals in electric fish.

Efficient sensory coding implies that populations of neurons should represent information-rich aspects of a signal with little redundancy . Recent studies have demonstrated that neural heterogeneity in higher brain areas enhances the efficiency of encoding by reducing redundancy across the population . Here, we investigate how neural heterogeneity in the early stages of sensory processing influences the efficiency of population codes. Through the analysis of in vivo recordings, we contrast the encoding of two types of communication signals of electric fishes in the most peripheral sensory area of the CNS, the Electrosensory Lateral Line lobe (ELL). We show that communication signals used during courtship (big chirps) and during aggressive encounters (small chirps) are encoded by different populations of ELL pyramidal cells, namely I-cells and E-cells respectively. Most importantly we show that the encoding strategy differs for the two signals and we argue that these differences allow these cell types to encode specifically information-rich features of the signals. Small chirps are detected and their timing is accurately signaled through stereotyped spike bursts, whereas the shape of big chirps is accurately represented by variable increases in firing rate. Furthermore, we show that the heterogeneity across I-cells enhances the efficiency of the population code and thus permits the accurate discrimination of different quality courtship signals. Our study demonstrates the importance of neural heterogeneity early in a sensory system and that it initiates the sparsification of sensory representation thereby contributing to the efficiency of the neural code.

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 P 249

A neural correlate of selective envelope coding in the electrosensory system.

Many sensory neurons respond to linear as well as nonlinear transformations of incoming sensory input. Perhaps the best-known example of this is the fact that auditory neurons that respond not only to the sound waveform but also to its time varying envelope. Simply speaking, the envelope can be viewed as the smooth curve connecting successive maxima in the stimulus waveform and represents the “instantaneous amplitude of variance”. Envelope information is often necessary for speech perception in humans, and is used in stereopsis and in other forms of visual perception. For example, people easily perceive both the frequency of a sound as well as its amplitude modulations (i.e. the envelope). In the visual system contrast-based visual contours, another form of envelope, are also readily perceived and used in motion processing. Weakly electric fish detect the amplitude modulations of their self-generated electric organ discharge caused by prey and conspecifics through an array of electroreceptive neurons on their skin. Recent studies have shown that envelopes (i.e. amplitude modulations of the amplitude modulation) can occur in natural situations and that hindbrain electrosensory neurons respond to these envelopes. Here we show that midbrain electrosensory neurons can display strong responses to the envelopes but weak responses to the stimulus waveform itself. We note that this can occur even when both the stimulus and the envelope waveforms have overlapping temporal frequency content. Through a combination of electephysiological recordings from midbrain and hindbrain electrosensory neurons as well as mathematical modeling. We reveal the mechanism by which midbrain neurons combine inputs from hindbrain neurons in order to generate this selectivity. We propose that such mechanisms are widely used in other systems and may explain envelope selectivity observed there.

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P 250

Low-frequency envelope avoidance in weakly electric fish

Envelopes are low-frequency modulations of high-frequency carrier signals. In active electrosensation, envelopes may provide information about moving objects and prey, but might be subject to detrimental interference when fish are near conspecifics. Depending on the relative electric organ discharge (EOD) frequencies of each fish, low-frequency envelope modulations can emerge due to complex beating patterns. We tested the hypothesis that weakly electric fish avoid such envelopes by changing the frequencies of their EOD. In recordings made in a natural habitat (Napo River; N=15) we found little or no power in low-frequency envelopes (<15-20 Hz). Recordings made in laboratory tanks with groups of conspecifics are consistent with these observations from the field. Using artificial stimulation of individual fish we have preliminary evidence for an "envelope avoidance behavior". Fish (N=4) were tested with pairs of sinusoidal stimuli designed to generate low-frequency envelopes. The fish adjusted their EOD frequencies to change the lower-frequency envelopes resulting from interference between the stimulus and their own EOD. For example, when presented with sine waves of differential frequencies (dF) -12 and +18 relative to the fish's initial EOD, some fish shifted their EOD frequency down by 2 Hz. This is despite the fact that the closer of the two dF signals – the one at -12 Hz – would be expected to elicit the extremely robust jamming avoidance response behavior and therefore drive the fish's frequency up (away from the -12 Hz signal). However, making this downward shift in the fish's EOD increased the lowest envelope frequency from 6 to 10 Hz. These data suggest that fish are able to detect and process envelope stimuli, and use this information to modulate their motor output, thereby avoiding low-frequency envelopes that may be detrimental to the processing of salient stimuli such as prey items.

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Segregating transient signals from background in weakly electric fish

Electrocommunication in weakly electric fish involves transient frequency rises, so called “chirps” that stand out against a continuous background signal. Midbrain neurons in these fish are highly responsive to the transient but inhibited by the background signal. Physiological and modeling data indicate that the strong responses to the chirp signal are caused by excitatory inputs that differ in their phase and time relation to the signal in such a way that they positively interfere during chirps. Most animal species have developed communication signals to which specific sensory neurons are well tuned [1, 2]. These must be discriminated from other environmental signals that could be produced by conspecifics. For example, a person is often able to detect his/her own name spoken by a person somewhere in a room against a background murmur caused by a party crowd. This situation is referred to as the Cocktail party effect [3] and requires discrimination between signal and background. A similar situation occurs during social interactions of wave-type weakly electric fish. These fish perceive the electrical properties of the environment by sensing the perturbations in a self-generated electric field. In wave-type fish the electric organ discharge (EOD) causes a quasi-sinusoidal continuous electric field. When two conspecifics are in close proximity a beat pattern can be observed due to the interference of their electric fields. This background signal can be interrupted by so called “chirps“, which are transient frequency rises in the beat caused by an active transient increase in the EOD frequency of one of the two fish. These chirps are highly stereotyped and are typically produced during aggressive or courtship encounters [4]. Moreover, recent behavioral evidence suggests that these fish can discriminate these chirp signals from the background beat [5]. Here we provide evidence for a neural correlate of signal-background segregation in the midbrain of these fish. Specifically, we show that some neurons within the Torus Semicircularis (equivalent to the Mammalian inferior colliculus) can strongly respond to chirps and weakly or not at all to the background beat. Intracellular recordings from these neurons revealed that, while they are inhibited in the presence of a beat stimulus, they displayed biphasic responses in their membrane potential suggesting that they receive at least two out-of-phase periodic, beat related, inputs. We present a phenomenological model that explains how such inputs can interfere either constructively or destructively depending on the phase relationship between the chirp and the beat. The model correctly predicts the selectivity of toral neurons to specific chirp shapes. We confirm these findings with a Hodgkin-Huxley model that includes tonic inhibition and realistic synaptic input currents. Our results provide general cellular mechanisms by which a downstream neuron combines afferent input in order to generate selective responses to sensory input and may apply to other species. 1. Rieke, F., Bodnar, D. A. and Bialek, W., Proc Biol Sci 262, 259-265 (1995). 2. Machens, C. K. et al., J Neurosci 21, 3215-3227 (2001). 3. Cherry, E. C., J Acoust Soc Am 25(5), 975-979 (1953). 4. Zakon, H. et al., J Physiol Paris 96, 451-458 (2002). 5. Hupé, G. J. and Lewis, J., J Exp Biol 211, 1657-1667 (2008).

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P 252

Are there Electric Colours? Exploring the perceptual space of Gnathonemus petersii.

The weakly electric fish G. petersii orients itself using a self-generated electric field (EOD). Two types of receptor cells detect changes in the EOD caused by objects: A-cells are sensitive only to changes in EOD-amplitude; B-cells respond to amplitude and waveform-distortions caused by capacitive objects. The initial step of neural processing beyond mere signal-transduction occurs in the Medulla, where information of both receptor cells is handled separately. ELL neurons project to the Nucleus lateralis, the first station where information of both cells converges. It is unknown which sensory parameters are extracted here. We speculate that neurons in NL merge information of A- and B-cells to determine objects‘ complex impedance. To gain further insights into sensory processing we investigate responses to “virtual” resistive or capacitive objects. Additionally we conducted behavioural tests. Fish had to decide between virtual objects of the same and different electric colours. Our results show that fish don‘t respond differently to objects of certain electric colours, suggesting that this parameter may not be used for object discrimination. A natural behaviour (novelty response) was exploited and does add support to this conclusion. In addition, these experiments reveal that the amplitude and the probability of the novelty response depend on the stimulus history and on stimulus contrast, but not on the absolute EOD amplitude. With regard to the hypothesis of electric colours, novelty experiments using an abrupt change of the electric properties of virtual electrical objects were well responded to. However, fish responded equally well to stimuli of identical colour and to changes that were between colours. Thus our data does strongly argue against the perceptual presence of colour lines in weakly electric fish.

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Engelmann, Jacob

P 253

Responses of sensory neurons to the existence of real objects reveal a mixed coding strategy in the brain of a weakly electric fish, Gnathonemus petersii

Encoding of certain stimulus parameters by the brain is an important prerequisite for the execution of adequate behaviours of an animal. The scope of this study was to determine coding strategies of sensory neurons in the brain of Gnathonemus petersii regarding rate and temporal coding. Therefore we presented either a point stimulus or real objects made of different materials in the receptive field of a neuron. With respect to the responding properties we determined E- and I-units. Nearly all of the recorded neurons responded to the presence of an object. In most cases, the object caused amplitude modulation was represented by a mixed rate-latency code (10 E-units, 16 I-units). Encoding exclusively by rate or latency occurred, but with less frequency (rate: 5 E-units, 3 I-units; latency: 2 E- units, 3 I-units). Additionally, we were interested in the coding range of electrosensory neurons. Therefore we analysed the responses of electrosensory neurons to variable stimulus amplitude intensities. Most of them (56.1%) encoded the stimulus amplitude in an all-or-non fashion when reaching the sufficient amplitude. Linear encoding was observed in a total of 29 recorded neurons. The proportion of linear encoding was highest for the Schnauzenorgan and decreased towards the trunk of the fish. However, neurons responsible for the Schnauzenorgan showed the narrowest coding range, whereas neurons responsible for the trunk had the broadest coding interval due to the basic signal amplitude. This indicates different coding duties for neurons based on their somatotopic location within the electrosensory lateral line lobe of G. petersii.

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Response of neurons in the electrosensory lateral line lobe of the weakly electric fish Gnathonemus petersii to real objects presented at different distances

The weakly electric fish Gnathonemus petersii explores its environment by active electrolocation, which enables it to detect nearby objects and discriminate between different object parameters. The focus of this study is to understand how this is achieved neuronally at the first medullary brain nucleus, the electrosensory lateral-line lobe (ELL). We examined the receptive fields (RF) of ELL neurons and investigated which impact an object's distance has on the encoding of the electrical images. Previous studies have characterized RF by aid of local electrical point stimuli in absence of the animals own electrical signal (EOD), neglecting both temporal and spatial aspects of the neuronal network and the electrical images of objects. Here, we used whole body stimulation to simulate the natural electric field of the fish and presented real objects (a plastic or a metal cube of 8 x 8 x 8 mm3) within the RF of the neurons. With extracellular recordings of single cells, we characterize how object properties like resistivity are encoded. Real objects cause local modulations of the EOD called "electric image", which are perceived by cutaneous electroreceptors and forwarded to the first stage of sensory processing, the ELL. With both object types, we determined the RF organization by placing the objects close to the fish and subsequently raising the distance to 5 and 10 mm to determine the range of electrolocation. Both E- and I-units were investigated using spike rate and first spike latency as measures. Results of neurons in the medial zone (MZ) and dorsolateral zone (DLZ) of the ELL were compared. In addition, we characterized the reciprocal anatomical connections between these zones. Our data suggest that receptive fields of ELL neurons are larger and more complex when real objects are presented compared to measurements with point stimuli. Furthermore, we found that neurons still responded to objects presented at distances larger than 5 mm from the fish.

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GABAergic Inhibition Shapes Interpulse Interval Tuning in the Electrosensory System of Mormyrid Electric Fish.

Mormyrid electric fish communicate using an electric organ discharge (EOD). By varying interpulse intervals (IPIs) between EODs, mormyrids produce a diverse repertoire of signals that play important roles in social behavior. Recently, we demonstrated that initial processing of IPIs occurs in the posterior exterolateral nucleus (ELp). ELp neurons exhibit distinct patterns of IPI tuning, including low-pass neurons, high-pass neurons and band-pass neurons. In the current study, we addressed the role of GABAergic inhibition in filtering temporal information by ELp neurons. Using immunohistochemistry, we demonstrate the existence of GABAergic cells in ELp. Using a new in vitro brain slice preparation, we made whole-cell current-clamp recordings from ELp neurons while stimulating afferent inputs to ELp with IPIs ranging from 10 to 100 ms. We recorded from 55 ELp neurons, including low-pass (n=14), high-pass (n=16), band-pass (n=12), all-pass (n=5) and band-stop (n=5) neurons, as well as neurons with more complex IPI tuning (n=3). Bath application of GABAA receptor antagonists (e.g. bicuculline, picrotoxin or gabazine) led to a significant increase in the amplitude of PSPs (n=32; $p<0.001$); 18 of these neurons showed complete washout ($p<0.001$). No differences were observed between pre-drug and vehicle alone (n=13; $p>0.88$). Blocking GABAergic inhibition caused a shift towards high-pass tuning: all high-pass neurons became more sharply tuned (n=11; $p<0.01$), and 15 out of 21 remaining neurons switched to high-pass tuning. Our results suggest that GABAergic inhibition plays a critical role in IPI processing in the mormyrid electrosensory system. The shift towards high-pass tuning after blocking inhibition appears to result from the summation of EPSPs at short IPIs. We propose that the primary role of inhibition is to counteract the effects of EPSP summation, and that differences in the strength of inhibitory input between neurons establishes different patterns of IPI tuning.

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P 256

Electric communication and social behaviour during various situations in a group of weakly electric fish, Mormyrus rume (Mormyridae, Teleostei)

African mormyrid fish generate and receive low-voltage short-duration electric pulses (electric organ discharges, EODs) in order to sense objects in their environment and for electrocommunication. By using their tuberous electroreceptor-organs to sense the EODs of conspecifics fish can detect each other's presence. Analysing the highly stereotyped EOD waveform, the fish can recognise the species and the sex of the sender. In addition, inter-discharge intervals (IDIs) are variable and enable mormyrids to communicate with each other during social interactions. In contrast to other species of weakly electric fishes, Mormyrus rume appears to be a social species, where especially young fishes often swim together, and rarely engage in antagonist interactions. We investigated electrocommunication behaviour in a group of 5 M. rume during various situations such as foraging, resting, and fighting. In each situation we looked for behaviourally caused characteristic temporal patterns of IDIs. Depending on the positions of the fishes in the experimental tank, it was possible to assign each recorded EOD to an individual freely swimming sender of the group. The analysed sequences showed social behaviour during group foraging, which was also identifiable in highly regularly IDI-patterns. In addition, 2-5 M. rume, swimming together during foraging, showed temporarily synchronized discharge patterns. During resting we found differences in IDI-patterns of fishes in a group compared to isolated fish. Resting M. rume produce fewer signals in a group and appeared to be calmer then fishes resting in isolation. Antagonistic encounters, during while a fish was chasing another one, were characterised by extremely short IDIs which slowly increased in duration. Until now, this pattern was not found in other social situations but for a better understanding, analysing of more sequences and identification of more specific communication-patterns are required.

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P 257

Towards autonomous large scale recordings of natural behavior of weakly electric fish

Weakly electric fish are a well established model system for studying sensory processing in vertebrates, providing great electrophysiological accessibility as well as a concise behavior. These preconditions make it one of the few model systems where electrophysiological data have been successfully discussed in the context of behaviorally relevant stimuli. In order to better understand tuning properties of the electrosensory system, data on the statistical structure that characterizes natural communication signals is needed. However, most behavioral data have been acquired under very restricted lab conditions. We present a novel method that allows to monitor the movement, electric organ discharge (EOD), and communication signals of individual weakly electric fish in their natural habitat. In our proof-of-concept study, we use a setup composed of an evenly spaced grid of 16 electrodes covering up to 9 m2. In a first step, we identify individual fishes by spectral analysis. Fitting a 3D dipole model to the data yields the fishes positions, orientations and EOD strengths. In a second step, the individual fishes EOD is extracted for analysis, e. g. detection of chirps. In a third step, the EOD of all recorded fishes in proximity are integrated at each fish''s position to construct the electric signals each fish receives in context of communication. For the first time, this new method allows to quantitatively analyze the interactions and communication of groups of weakly electric fish in their natural habitat. The method also provides powerful means to cover daily and seasonal changes in communication behavior as well as a novel framework for playback studies.

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P 258

Synaptic dynamics and plasticity of Purkinje cells onto their target cells in the mormyrid fish cerebellum

It is known that cerebellar Purkinje cells release the inhibitory neurotransmitter GABA to inhibit their target neurons located in the deep cerebellar nuclei (DCN). Purkinje cell axons also send collaterals back to the Purkinje cell layer, presumably to inhibit neighboring Purkinje cells. However, direct evidence on how Purkinje cells inhibit their target cells is still lacking. The present study was designed to examine how Purkinje cells inhibit their target cells. We chose a unique cerebellar model, the mormyrid electric fish cerebellum, in which cerebellar efferent cells (functionally equivalent to DCN cells of mammals) are intermingled with Purkinje cells in the cortex. Dual whole-cell patch-clamp recordings were performed in cell pairs of Purkinje cell and efferent or Purkinje cell in slice preparations and cells were identified morphologically after recordings. It was found that the synapses between Purkinje cells and their target cells can be classified into at least two types, weak synapse and strong synapse, based on the responses of target cells to Purkinje cell firing. At weak synapses, single Purkinje cell spikes hardly elicit any response in postsynaptic cells; however, if Purkinje cells fire spikes in bursts of ~20 ms at ¡Ý30 Hz, a group of IPSCs or IPSPs are induced in postsynaptic cells. In this case, IPSCs or IPSPs show clear delays and outlast the duration of presynaptic burst firing. At strong synapses, responses in postsynaptic cells to presynaptic Purkinje cell firing are highly reliable, and nearly each individual Purkinje cell spike, evoked or spontaneous, elicits an IPSC or IPSP in the postsynaptic cell. Thus, the responses of efferent cells and Purkinje cells to Purkinje cell firing are largely similar. In weak synapses, evoked burst firing alone at 0.5-1 Hz for ~2 min in presynaptic Purkinje cells lead to IPSP depression in postsynaptic cells, whereas pairing such presynaptic burst firing with postsynaptic spikes lead to potentiation in IPSPs in postsynaptic cells. These results indicate that synaptic dynamics of cerebellar Purkinje cells onto their target cells are variable and the inhibitory effects of Purkinje cells on their target cells can be plastic.
Supported by a grant from NSF IOS 0920672 to VH

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P 259

Electrolocation and electrocommunication pathways involving the telencephalon of Gymnotiform fish

Weakly electric fish use their electric organ discharge to locate themselves and to communicate with con-specifics. Although the coding of electric signals have been well explored in mid and hindbrain structures over the past 30 years, little is known regarding the integration and processing in higher order regions. Using biotinylated dextran amine we looked into the ascending and descending pathways to and from the telencephalon of the weakly electric fish Gymnotus carapo in order to identify the input of electrosensory information to the pallium, and the motor pathways that might allow telencephalic centers to influence behaviours. We found that the major input to the telencephalon comes from the preglomerular nucleus (PG) that projects to the dorsolateral and dorsomedial pallium. PG receives input from dorsocentral telencephalon (DC), nucleus tuberis anterior (TA), lateral hypothalamus, torus semicircularis dorsalis (TSd) and optic tectum (TeO). Throughout the vertebrates the TeO has been implicated in orienting responses towards to or away from a novelty stimulus. We hypothesise therefore that electrolocation information processed in TeO reaches the pallium through PG. On the other hand, electrocommunication information could be relayed to the pallium through two PG-projecting areas: the TSd and/or the TA, the latter being the target of nucleus electrosensorius neurons responsive to communication signals characteristic of courtship and aggression behaviours. Our hypothesis is that these electrocommunicaton signals are integrated with electrolocation signals from TeO within the pallium informing the animal the location of the communication signals. DC projections to electrosensory structures might influence pure electric behaviours while its projections to the ventral part of nucleus praeeminentialis, which processes ampullary information, might modulate behaviour linked to low-frequency electric discharge, such as defence/feeding, communication and/or parenting.

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P 260

Distribution of Muscarinic Acetylcholine Receptors in the brain of weakly electric fish

In order for an organism to interact appropriately with the environment, the brain has to select and process relevant sensory stimuli in a highly dynamic way. Processing properties have to be adjusted depending on conditions of the environment and the behavioral state of the organism. Various neuromodulators have been shown to be involved in shaping sensory information transmission by the brain. Acetylcholine (ACh) modulation, through muscarinic receptors (M), is a particularly widespread mechanism of controlling information transmission in various brain areas. Muscarinic receptors have been implicated in processes, such as attention, learning and memory, and enhancing neuronal responses to sensory stimulation in different sensory modalities. In the weakly electric fish, there is evidence of a cholinergic pathway acting in the electrosensory lateral line lobe (ELL) that suggests a role of ACh in the modulation of electrosensory information transmission. In this study we partially cloned three subtypes of muscarinic receptors (M2, M3 and M4) and used in situ hybridization in transverse sections of the brain of Apteronotus leptorhynchus to determine the expression patterns of those receptors. Positive label sites for the three muscarinic receptors were found over different brain regions devoted to the processing of various sensory modalities. The mRNA probes showed differential distributions but also overlapping presence of two or more receptors in particular nuclei. The expression patterns are in agreement with those of other species, with additional presence in specialized electrosensory regions including nucleus praeeminentialis, torus semicircularis, optic tectum and nucleus electrosensorius. The ELL region stained positive for M3 confirming the participation of ACh in different stages of electrosensory processing.

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Why knifefishes are shaped like knives

The role of mechanics in shaping body form is a fundamental issue in the study of movement. Fish are an ideal model system for this problem, since they have relatively simple shapes and low complexity of motion tasks compared to humans and other land animals. This makes them tractable for quantitative approaches where their shape is compared to a theoretical optimum. Among fish, knifefish have one of the more extreme body plans. The forward extension of an elongated ventral anal fin with which they move has pushed the anus all the way up to eye level. Through an interdisciplinary approach using a robotic knifefish, computational fluid simulations, and measurements from live fish, we show that this unusual body shape maximizes forward propulsion from the anal fin. Remarkably, an unrelated fish that has the fin flipped to their topside shows a similar diagonal orientation, representing a convergence of biomechanical form and function. Our result simply and powerfully shows the permeating influence of mechanics on the evolution of the beautiful variety of animal forms around us.

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Shirgaonkar, Anup

Massachusetts Institute of Technology

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Artificial active and passive electrolocation

We explore the capabilities of an underwater robotic sensing platform which fuses signals from active electrosense and geomagnetic induction to navigate and recognize objects. The active electrosense subsystem is based on electrolocation in weakly electric fish, which emit a weak electric field to navigate and communicate. We extend a previous single-sensor design to five sensors. The geomagnetic induction subsystem is based on the electroreceptive ampullae of Lorenzini found in sharks, which may allow the animal to detect the orientation of earth"s magnetic field and ocean current velocities. Our implementation of this sensor enabled us to measure velocity with error below 1 cm/s. For a set of trials where the sensor was translated along a single axis of motion, with frequencies at or below 1 Hz and velocities at or below 4.0 cm/s, the standard deviation of velocity error was 0.08 cm/s in Earth"s magnetic field. In comparing sections of trials, we found error to be mainly related to acceleration magnitude and frequency rather than velocity. When we compare our overall voltage sensitivity at low frequencies to that found in sharks and scale for shark morphology, our results lend support to sharks" use of this modality to sense ocean current and travel velocity. When we combine the individual channels of the active electrosense system and the geomagnetic system using a statistical model (Bayesian filter) for estimating position or identifying nearby objects, we find significant improvements in precision and accuracy. The approach has several engineering applications, and help us better understand issues of sensor count, sensor density, and multisensory integration in the biological instantiation of active and passive electrosense.

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P 263

Electrolocation with Active Sensing in the Weakly Electric Fish

Weakly electric fish sense the change of electric potential caused by a neighbor object and it is believed that they estimate the distance of the object using a collection of electroreceptors. They have many electroreceptors on the skin surface and the readings of electroreceptors along the rostrocaudal axis are mapped into an electric image. It is well-known that the rate of maximal slope to maximal amplitude in the electric image, which is often called relative slope, is a direct cue for the distance of a target object, regardless of the size and electric property. Yet the measure of relative slope may experience inaccurate estimation of the distance because of noisy electric signals. Weakly electric fish normally produce a waveform of electric organ discharge (EOD), and there are two types of waveforms, pulse-type and wave-type. It has been reported that this waveform is related to communicate with conspecifics or handle jamming avoidance response. We suggest that the waveform of EOD can play a role of filtering the noisy signal in the electroreceptors. A sequence of varying levels of the EOD can produce temporal variation of electric potential at the electroreceptors. The cross-correlation between the EOD signal and the sensory afferent signals in the waveform can have an effect of denoising the perturbed electric signal caused by the neighbor objects and thus produce accurate relative slope in the noisy environment. As a result, the weakly electric fish can localize a target object more precisely. The cross-correlation process might be a possible mechanism for the localization in the weakly electric fish. In addition, spatiotemporal processing with active body movements, swimming back and forth, or bending the tail can help provide another aspect of electric sensor information and electrolocation features.

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P 264

Two modes of information processing in the electrosensory system of the paddlefish, Polyodon spathula

Paddlefish (Polyodon spathula) use their passive electrosensory system for the detection of their prey, small water fleas. With anatomical and electrophysiological techniques, we investigated how electrosensory information is processed in the brain. Primary afferent fibers innervate a single area in the hindbrain, the dorsal octavolateral nucleus (DON). Secondary neurons in the DON project then to the mesencephalic tectum and to two areas in the dorsal tegmentum, the torus semicircularis, and the lateral mesencephalic nucleus. We found here that the projections to the tectum and dorsal tegmentum arise from two different populations of neurons in the DON. Cells in the anterior DON project to the contralateral tectum whereas cells in the posterior DON project to the tegmentum, bilaterally. These cells are one order of magnitude more sensitive than the cells projecting to the tectum and respond better to lower frequencies. This makes them better suited to detect prey at a distance when signals are weaker and have lower frequency components. In contrast, DON cells projecting to the tectum can work in a proximity mode only, when the prey is close to the receptors in the skin. In this mode, spatial resolution is high and electrosensory information can directly converge with visual information in the tectum to guide prey capture behavior. This is the first report of two modes of information processing in the octavolateral system and may help to understand the functional role of the torus semicircularis as opposed to the tectum.

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Physiology and morphology of visual and electrosensory units in the tectum opticum of the paddlefish (Polyodon spathula)

The paddlefish is a passive electrosensitive fish, which is endemic in the Mississippi river system. The paddlefish is particularly sensitive and uses its oversized rostrum to detect the tiny electric fields of its primary prey, daphnia. Neuroanatomical studies showed that the primary brain area for processing the electrosensory input is the DON (dorsal octavolateral nucleus) in the hindbrain. Information is then relayed to the midbrain. Here, the tectum is the major integrative center that organizes prey capture. We have investigated response properties of visual and electrosensory units in the tectum. Intracellular recordings and dye injections were made in 67 cells from 16 paddlefish. Stimuli were light flashes, a moving shaddow, and weak sinusoidal electric fields. Physiologically three types of cells can be distinguished by their different responses to the stimuli: electrosensitive cells, visual sensitive cells and visual-movement sensitive cells. Within these groups different responses types can be distinguished. Electrosensory cells were mostly phase coupled to the sine wave, but many of them lack phase coupling and either increase or decrease their spike rate during stimulation. Visual units show either a tonic increase or decrease of spike rate or show phasic responses to the ON and/or OFF slope of the light pulse. The three different cell types also showed distinct morphology. Visual and visual-movement sensitive cells are located in the upper or middle layers of the tectum and have large dendritic trees which are spread over a wide area and are mostly multipolar. Electrosensitive cells are located in the middle or lower layers, have smaller dendritic trees and are mostly monopolar. Our data suggest that the visual and the electrosensory systems are working independent of each other. The electrosensory system may initiate the prey catching behavior while the visual system may be responsible for predator avoidance.

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P 266

Making Sense of Electro-sensitivity in Crayfish

It is puzzling that, in spite of investigations by many researchers (1) there are so few examples of electro-sensitivity in the invertebrates (2,3,4). We recently discovered electro-sensitivity in two Australian crayfish (Cherax destructor and Cherax quadricarinatus) that fall within the range described in other electro-sensitive animals and within the range of signals, both animate and inanimate, found in freshwater (5). We showed that the animals can detect low level electrical signals by demonstrating that they "pay attention": they change the behaviour in which they are engaged when the signal is played. During our extended study of the crayfish electro-sense we were encouraged by colleagues and referees to investigate why the animals evolved this sensitivity. In this presentation we describe some of the other experiments we conducted, including T-mazes, chemical co-stimuli and conditioning experiments, none of which appeared to resolve the importance of this sensitivity for these crayfish in the wild. (1) Bullock, T. H. J. Exp. Biol. 202, 1455-1458 (1999) (2) Weissberg, M.J., Levett, S.J., Steullet, P., Edwards, D.H. & Derby, C..D. Soc. Neurosci. Abs. 22, 1078 (1996) (3) Patullo, B. W. & Macmillan, D. L. Curr. Biol. 17, R83-R-84 (2007) (4) Steullet, P., Edwards, D. H. & Derby, C. D. Biol. Bull. 213, 16-20 (2007) (5) Patullo, B.W., Macmillan, D.L. J. Exp. Biol. 213:651 (2010)

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Learning & behavioral plasticity

539 **P 267**

Social environment influences performance in a cognitive task in natural variants of the foraging gene

The importance of how social environment affects behavior has recently received increased attention. In Drosophila, natural genetic variation in the foraging gene, which encodes for a cGMP-dependent protein kinase (PKG), affects the foraging activity of larval and adult flies. Sitters (fors) tend to be more sedentary and aggregate within food patches whereas rovers (forR) have greater movement within and between patches of food. Additionally, rovers and sitters vary in their performance on a number of cognitive tasks. We hypothesized that these variants would also differ, in a classical olfactory conditioning test, depending on whether they were in groups or alone. Individual performance was affected by PKG activity. In sitters, but not in rovers, the acquisition of information was facilitated by the social interaction (being in a group). In rovers, but not in sitters, the type of social interaction (being with other rovers or with other sitters) affected learning and memory. Also, naïve individual rovers tended to follow groups of conditioned sitters but not groups of conditioned rovers. Our results suggest that for mediates some social aspects involved in learning and memory in Drosophila melanogaster. Also, the traditional way of studying olfactory learning with groups may not be a good indicator of individual performance.

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P 268

Olfactory learning and memory in the larvae of Drosophila melanogaster

The larva of Drosophila melanogaster has a nervous system much simpler than that of the adult; it can be trained to avoid odours associated with electric shock. We have developed a paradigm of electroshock conditioning in the larvae that yields high learning indices similar to those one in the adult. This has enabled us to distinguish between different memory phases and study their kinetics. Three components of memory curve obtained after Scatchard analysis; short term (STM), middle term (MTM) and long term (LTM) which display distinct characteristics in their formation and decay kinetics. Analysis of leaning retention in mutants' rutabaga, dunce, radish and amnesiac are in agreement with our analysis. These phases of memory are differentially sensitive to memory ablating agents and are not serially connected. In order to find out biochemical correlates of olfactory learning, differential 2D gel electrophoresis has been carried out. Spots which show changes in expression have been sequenced by Mass Spectrometry. Proteins which over expressed due to learning have been broadly classified as receptors and channels which include 3 olfactory and 2 gustatory receptors and an olfactory binding protein. The second category is signaling molecules like protein kinases and other post translational modifications. The last category is proteins of unknown functions. One of the olfactory receptors, Or45a appears to make a novel projection in an adjacent glomerulus. The volume of the glomerulus Or45a in the antennal lobe shows approximately four fold increase upon training. There is also an additional enlargement at the entry of Or45a into the larval antennal lobe.

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Can flies learn complex olfactory associations?

Drosophila are capable of learning a robust behavioural response of avoidance to an odour that has been paired to an aversive stimulus, such as shock. A considerable volume of reseach has investigated the brain regions, underlying circuitry and genetic control involved in such associations, but this is usually based on straightforward elemental learning tasks in which one odour is reinforced and a second odour is not reinforced (A+ B-). In nature it is more likely that invertebrates are presented with combinations of stimuli (e.g. fruits or flowers combining several odours and colours) which would need to be processed appropriately to extract the relevant cues. We have limited understanding of the fly's ability to solve such complex discrimination problems. For example, a compound stimulus (AB) may be comprised of two elements (A and B), but is it processed simply as the sum of the two components, or is the compound somehow distinct? Our aim in this study is to systematically investigate the fly's ability to learn complex discriminations involving compound olfactory stimuli associated with shock. We found that flies could learn to distinguish binary mixtures (AB+ CD-), including overlapping mixtures (AB+ BC-, i.e., discrimination learning). They could learn positive patterning (AB+ A- B-) but could not learn negative patterning (A+B+ AB-) or solve a biconditional discrimination task (AB+ CD+ AC- BD-). Learning about the elements of a compound (AB+) was not affected by prior conditioning of one of the elements (A+ AB+): flies do not exhibit blocking in this task. They also do not exhibit sensory preconditioning. We compare these results with the predictions from simulation of several well-known theoretical models of learning, and find none are fully consistent with the overall pattern of observed behaviour. However, the results can be explained under some reasonably simple assumptions about the underlying neural mechanisms.

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A NEW BEHAVIOURAL ASSAY FOR AVERSIVE VISUAL LEARNING IN DROSOPHILA MELANOGASTER

To understand neurobiological mechanisms for visual perception and memory, we developed a new conditioning assay for aversive visual associative learning in adult flies. We designed a setup based on an existing appetitive conditioning assay. Flies are trained en masse to associate one of two different visual stimuli (e.g. blue and green light) with electric shock punishment. In the test phase, both punished and control visual stimuli were presented, and the difference in flies" visual preference was measured as associative memory. The trained flies showed significant conditioned avoidance of a punished cue. We analysed critical parameters for the formation and expression of visual memory, such as training repetition and strength of reinforcement. Since this assay uses the same visual stimuli as in appetitive conditioning and yields consistent conditioned behaviour, we can directly compare appetitive and aversive visual memories.

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Visual Place Learning in Drosophila

Many insects use visual landmarks to precisely locate their nest, prey, or foraging area. While this behavior has been extensively studied, an understanding of the neural basis for insect place memory has been hampered by the lack of reliable tools to probe invertebrate brains. Recent advances in Drosophila molecular genetics make Drosophila melanogaster well suited for investigating the neural correlates of behavior. While the Drosophila genetic toolkit makes fruit flies an appealing organism to work with, the extent to which flies use vision to navigate and remember specific locations has been unclear. In our current studies, we show that flies do, in fact, show a robust ability to learn spatial locations. To test Drosophila for place learning, we have designed a visual place learning assay inspired by the Morris Water Maze. Rather than use water, we use heat as the aversive stimulus. To precisely control the thermal environment, we developed a thermoelectric module (TEM) array composed of 64 individually addressable 1-inch2 peltier tiles arranged in an 8x8 grid. This array forms the floor of our test arena and a LED display is positioned around the circumference to deliver visual panoramas. To test place learning in flies, we set 63 of the tiles to an aversive warm temperature and set a single tile to a preferred cool temperature. Over successive trials, flies learn to rapidly and reliably locate this cool spot using peripheral visual cues presented on the LED display. Importantly, this improvement is critically dependent on the visual panorama containing spatially distinct visual patterns. Using this assay and Drosophila molecular-genetic tools, we have identified subsets of central complex neurons required for successful place learning.

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Pavlov’s Fly â€“ auditory conditioning in Drosophila.

The mechanisms that underlie learning and memory have long benefited from studies in the fruit fly, Drosophila melanogaster. The dominant learning paradigm in fruit fly studies is classical conditioning using olfactory stimulation as the training cue. We investigated whether audible 400 Hz sinusoid tone can serve as sensory cue to drive classical reflex conditioning, sensu Pavlov. We used a neutral--non-punishing--sound cue and coupled it with the fly"s natural reflex to extend its proboscis, when feeding. Wild type Canton-S-5 strain exhibits robust acoustic learning across a range of relevant intensities.We measure the lower threshold which wild type can hear. Finally, we deploy a powerful and robust statistical methodology for learning assessment, namely generalized estimating equations that is conventionally employed by researchers in the mammalian field, but is novel for fly researchers, who use a simple but less robust "learning index" ratio measure. The significance of our findings for drosophilists is to open up a new sensory modality, the auditory sense, for the study of learning; this is significant because the great majority of fly learning is based on the chemical senses of taste and smell. Our finding can be used to screen for new auditory mutants in females and the physiologically recording from the JO can be used to determine whether any deficit in hearing is peripheral or central. Acoustic stimuli are easier to quantify and manipulate for learning than chemical cues, and if compounded with chemical cues, it can extend learning studies to multiple modalities.

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P 273

Visual pattern memory in Drosophila is different for "novelty learning" and heat conditioning

"Novelty Choice" is a visual learning paradigm without any obvious reinforcer. Instead it relies on the fly's spontaneous preference for suddenly appearing new patterns compared to the existing old ones[1]. In the so-called standard learning experiment in the flight simulator the fly forms a classical association between a pattern parameter and heat punishment, and uses this memory trace to later avoid the corresponding pattern. In the novelty paradigm the fly forms a memory trace of the old pattern even without heat punishment (incidental memory). The test for memory retrieval is the same in both paradigms. We have found that rutabaga mutants (rut) have no learning defect in the novelty paradigm. It is known that associative learning and memory require an intact Rut protein, a type-1 adenylyl cyclase considered to be a coincidence detector between US and CS[2]. So far five visual parameters: Height, Size, Vertical Compactness, Edge Orientation and Color[3] can be identified in the standard experiment. Surprisingly it appears that not all of them can be "learned" in the novelty paradigm. In the present study we show that of the tested two parameters Height and Edge orientation, novelty choice can be shown only with the former and not the latter. Like standard learning novelty learning seems not to require the mushroom bodies while the ellipsoid body (EB) seems to be necessary for both tasks. We also found that ignorant (ign) mutants are defective in novelty learning. Our latest results show that Ring neurons of the EB may be involved in novelty learning as they are in standard learning[4]. In contrast, the F5-neurons in the Fan-shaped body (FB), which have been shown to be required for the standard learning experiment in the flight simulator[5] may be dispensible for novelty learning.
References: (**1**) Dill, M. , Heisenberg, M. (1995) Phil Trans Roy Soc Lond B Biol Sci. **349**: 143-52. (**2**) Zars, T. Wolf, R. Davis, R. Heisenberg, M. (2000) Learn Mem. **7**: 18-31. (**3**) Tang, S. Wolf, R. Xu, S. Heisenberg, M. (2004) Science **305**: 1020-2. (**4**) Pan, Y. Zhou, Y. Guo, C. Gong, H. Gong, Z. Liu, L. (2009) Learn Mem **16**: 289-95. (**5)** Liu, G. Seiler, H. Wen, A. Zars, T. Ito, K. Wolf, R. Heisenberg, M. Liu, L. (2006) Nature **439**: 551-6.

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Conditioned behaviour in Drosophila larvae: to what do they respond?

We developed a new paradigm to study aversive classical conditioning in Drosophila larvae. Larvae receive one odour (A) together with a high-frequency substrate vibration as punishment. Another odour (B) is presented without punishment. In a subsequent test, the larvae prefer the previously non-punished odour B. I will present some of the basic parametric features of this type of learning. Also, I show that the testing conditions are critical for the expression of conditioned behaviour. Specifically, conditioned preference for the non-punished odour is only seen when the testing situation is "bad", i.e. if the larvae can expect that going towards the odour B might improve the situation. If the testing situation is "good", odour B does not signal any opportunity for improvement, and hence no conditioned behaviour is expressed. This seems to reflect a principle organization of conditioned behaviour according to its expected outcome ("improvement": Hendel et al, 2006). I will discuss in particular how this concept bears upon the question whether behaviour is better viewed as spontaneous action or as a stimulus-induced re-action. Having now two aversive paradigms at disposition (one using gustatory punishment by salt or quinine, and the current paradigm using substrate vibration), we can use the genetic tools available in Drosophila to understand the principles of how this comes about.

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Modelling the neural circuits of insect olfactory learning

Establishing structure-function relationships is a fundamental issue in neuroethology. Modelling approaches allow for systematic exploration of these relationships (in simulation or in more realistic environments using robots). Here, we model network interactions in the neural circuitry of the insect antennal lobe and mushroom body, to investigate their role in olfactory learning. Using single compartment neuron models, we systematically investigate the ability of this distinct neuro-architecture for decorrelating responses to odours, and how this might support learning of complex discriminations involving compound stimuli (including binary and overlapping mixtures, negative and positive patterning, biconditional discrimination, blocking, sensory preconditioning). We compare these results with experimental data from odour-shock conditioning in flies, and the predictions from several well-known theoretical models. We also present a hardware model of this circuitry, implemented on a Field-Programmable Gate Array, which can control a robot in closed-loop learning experiments.

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619 **P 276**

Rescue of the dunce learning mutation.

The second messenger cAMP plays a pivotal role in memory formation and its concentration is tightly regulated by adenylyl cyclases (ACs) and phosphodiesterases (PDEs). It is these "checkpoints" of cAMP that are affected in the mutants dunce (a PDE) and rutabaga (an AC), originally isolated due to their poor learning performance. Functional rescue of the rut-dependent learning defect identified rut-AC function within Kenyon Cells (KCs) of the Mushroom Bodies to be sufficient for odor learning, placing cAMP dependent plasticity at the KC layer at a central position for this type of behavioral plasticity. Here, we have performed functional mapping of the neuronal circuit that requires dnc-PDE function. We identified two neural layers of the olfactory pathway, GABAergic local neurons (LNs) at level of the antennal lobes and KCs at level of the Mushroom Bodies to require dnc-PDE for odor learning. At either layer dnc-PDE functions in a non-redundant fashion and together LNs and KCs define a minimum circuit where dnc-PDE function is necessary and sufficient for odor learning. The discrepancy in overlap between the requirement for either dnc-PDE and rut-AC function on level of neuronal circuits lead us to investigate performance of rut-dnc double mutants and we show that either mutation affects a distict aspect of odor memory. This result shows that two distinct forms of cAMP dependent plasticity are involved in odor learning: One rut-AC depenent mechanism localized at the KC layer, and a dnc-PDE dependent mechanism localized at the level of KCs and LNs.

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P 277

Multiple signaling thresholds contribute to aversive odor memories. A parametric analysis of US quality and quantity.

Temporal aspects of the training procedure have a strong impact on memory stability. Within the Drosophila aversive odor conditioning paradigm a memory is formed due to association between an odor cue (CS) and electric shock punishment (US). For nearly 25 years this paradigm has remained unchanged and the US is represented by a train of individual shock pulses applied in fast succession. The resulting memory is composed of distinct phases typically refered to as short-term memory (STM), anesthesia-resistant memory (ARM) and anesthesia-sensitive memory (ASM); those can be se-parated due to kinetic and/or functional aspects. In order to differentiate between the molecular mechanisms supporting those memory phases we have investigated the impact of US quality and quantity by varying number and voltage of shocks experienced during the association procedure. Our results revealed that memory phases are differentially affected by variation of US properties, thus suggesting different molecular mechanisms to be involved: While formation of STM is a continuous process, acquisition of ARM and ASM are discontiuous. ARM is unaffected by either US quality or quantit and represents a `snapshot` memory with instantaneous aquisition. In contrast, ASM formation is dependent on two consecutive US exposures and requires interaction between PKA and A-Kinase-anchoring-proteins (AKAPs) in Kenyon Cells of the Mushroom Bodies. Actually, we determine the role of separable cAMP pools in formation of these distinct memory phases and continue to define kinetic aspects of consecutive stimulation. Finally, we will use Photoactive Cyclases (PACs) to manipulate in-tracellular cAMP levels and determine their functional implication for ASM formation.

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622 **P 278**

Where does Potein-Kinase A support odor memory? Functional memory maps based on a RNAi knockdown approach.

PKA is the textbook example of a cAMP downstream target and it is involved in synaptic and behavioral plasticity across the animal kingdom. In Drosophila, the most abundant catalytic subunit of PKA is encoded by the DC0 gene and appropriate mutants show poor odor learning. However, the neuronal substrates are unknown. To map the neuronal substrates of DC0-PKA function, we have used ectopic knockdown of DC0 and analysed the effects on reward- and punishment learning. Our results revealed a complex involvement of PKA signaling in memory maturation with multiple dissociations between reward and punishment learning. Actually, we define the functional correlation between PKA and separable cAMP pools by simultaneous manipulation of DC0 and either rut-AC or dnc-PDE. As both forms of behavioral plasticity, i.e. reward and punishment learning, share common elements on level of neuronal pathways and molecular signaling, this comparative analysis is ideally suited to define signaling divergences along the cAMP-PKA pathway.

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640 **P 279**

The what and where of operant self-learning mechanisms in Drosophila

In most operant learning experiments, two biological processes take place, world-learning (the process assigning value to sensory stimuli) and self-learning (the process assigning value to a specific action or movement). Using tethered Drosophila at the torque meter, the two processes can be separated and studied independently. Manipulation of synaptic plasticity via the cAMP pathway disrupts world-learning but does not affect self-learning. Conversely, inhibiting PKC activity affects self-learning, but leaves world-learning intact. In order to decipher the molecular bases of self-learning, we use the genetic tools available in Drosophila in combination with behavioral tests. Because the expression of an inhibitor of PKC interferes with self-learning, we have started to screen the available non-lethal mutants of the different PKC isoforms for deficits in self-learning. Interestingly, self-learning of the tested mutants was unaffected. In order to overcome potential compensatory mechanisms among the different PKC isoforms during development, we are now using an RNAi-induced downregulation of specific PKC isoforms only during adulthood (via the Pswitch system) to test their role in self-learning. In addition to PKC, we also investigate the involvement of the fly orthologue of the human FOXP2 gene. Both a P-Element insertion and RNAi-mediated knockdown of the last exon of the Drosophila FoxP gene did not lead to alterations of the gross brain anatomy, nor to an impairment in operant world-learning. However, both fly strains were impaired in operant self-learning. These results suggest a specific involvement of the Drosophila FoxP gene in the neural plasticity underlying operant self-learning but not other forms of learning. To investigate the effects of RNAi knockdown and P-Element insertion on FoxP abundance and localization in the fly central nervous system, we have initiated an analysis of FoxP expression patterns on the mRNA as well as on the protein level.

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P 280

Age-related memory impairment and its pharmacological rescue in the cricket

Age-related memory impairment (AMI) is reported in many animals including insects. We have found that crickets *Gryllus bimaculatus* have excellent learning and memory capability. Here, to investigate whether AMI is observed in the crickets, we trained male crickets of different ages with one of three multiple-trial appetitive conditionings (olfactory, visual pattern or color conditioning) and tested their preference after training. Crickets aged three weeks after the final molt (3-week-old crickets) exhibited normal levels of retentions at 1 hr after all three conditioning types but they showed significantly decreased levels of 1 day retention in comparison with 1-week-old crickets. These results indicate that AMI is observed in long-term memory but not in medium-term memory in olfactory, visual or color learning of crickets. Moreover, 3-week-old crickets injected with a nitric oxide (NO) donor, SNAP, or a cyclic GMP (cGMP) analog, 8br-cGMP, into the hemolymph before conditioning led to a significant level of 1-day retention, the same level as in 1-week-old crickets. For the first time in invertebrates, an NO donor and a cGMP analog were found to antagonize AMI, suggesting that the NO-cGMP signaling is important in brain aging processes.

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The role of pollen as a reward for learning in honeybees

Learning plays an important role in the exploitation of unpredictable food sources by honeybee colonies. Typically studies of appetitive learning have used sucrose solution as the unconditioned stimulus (US). However, bees forage for pollen as well as nectar and display clear preferences, suggesting they are able to distinguish between different pollen types. Unlike nectar, which is ingested and carried in the crop of foragers, pollen is stored in the leg corbiculae, and as yet it is unclear whether it might reinforce learning about floral features in the same manner as sucrose. The proboscis extension response (PER) paradigm was adapted to determine whether pollen can serve as the US in a classical conditioning task. Though bees were observed to extend the proboscis when the antennae were touched with pollen, we found no evidence that they learnt to associate this reward with the delivery of a neutral odour. Response to pollen itself was seen to decline over the course of the experiment. This decline was observed even when bees were prevented from consuming the reward, indicating that honeybees may use a pre-ingestive mechanism for detecting pollen compounds, which may in turn inhibit PER conditioning. When bees were trained under free flying conditions, however, they were able to associate a pollen reward with coloured stimuli, suggesting that the reinforcing properties of pollen vary according to behavioural context.

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P 282

Associative learning for the polarized light in the honey bee

Many insect species were suggested to use the polarization pattern for obtaining the accurate orientation during their navigation. Polarization vision in insect is mediated by the small area of the dorsal part of the compound eye, dorsal rim area (DRA). It, however, still remains unclear how the central nervous system processes the information of e-vector of the polarized skylight. Here we will show that a honeybee has a capability of learning of certain e-vector orientations by using a classical associative conditioning paradigm with the proboscis extension reflex (PER). An e-vector orientation as a conditioned stimulus (CS+) was associated with sugar water as an unconditioned stimulus (US), while another e-vector orientation (CS-) was not. Associative conditioning showed that the honeybees could discriminate CS+ from CS- after eight conditioning trials. Bees in spring exhibited higher learning score for CS+ than bees in autumn in spite of same colony□fs bees. Bees whose DRA was covered with a black paint could not learn CS+ whereas the animals could learned CS+ if the area of compound eye except for the DRA was covered. When the ultraviolet light (350 nm) was used for CS, bees could discriminate CS+ from CS- but not when the CS was blue (442 nm) or green (546 nm). Those results indicate that a honeybee can learn and discriminate e-vector orientations, suggesting that they could detect their flight orientation from the polarized skylight information during their foraging. Finally, by fixing their neck to stabilize their head through the experiments we examined whether a honeybee can discriminate two e-vectors without scanning the polarized light by turning its head, and found that a honeybee did discriminate between CS+ and CS-. This is the first evidence to show that the □ginstantaneous□h method is used in the polarization vision system of the insect brain, which requires no active scanning of the sky.

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P 283

Aversive conditioning in the honeybee

Honeybees are model organisms for the study of learning and memory. However, the study of learning and memory in bees has focused so far on appetitive learning and memory. Here we studied whether bees are capable of learning and memorizing about aversive events. We established a novel protocol for the study of aversive learning in the laboratory, the olfactory aversive conditioning of the sting extension reflex (SER). In this protocol, a harnessed bee, which responds with a sting extension to a noxious stimulation of a 7,5 V electric shock, learns that a neutral odorant anticipates the shock and starts exhibiting the SER to the odorant as a consequence of learning. We show that this learning is aversive as bees trained avoid afterwards the punished odorant when they can freely move within a Y-maze. Aversive learning leads to the formation of long-term aversive memories depending on protein synthesis that can be retrieved several days after training. Moreover, while reward is mediated via octopaminergic signaling, punishment is mediated via dopaminergic signaling within the brain. The relative insulation of these two neural modules determines that bees can master simultaneously aversive and appetitive associations. Such a hedonic modularity underlies the existence of different, uncorrelated behavioral syndromes within a hive, with some bees being more responsive to appetitive stimuli and others to aversive stimuli. Higher sensitivity to aversive punishment determines better aversive learning performances. This translates into task specialization with nectar foragers being more sensitive to the shock and learning better aversive associations, and guards being less sensitive (more tolerant) to the shock and therefore learning less well aversive associations. All in all, our studies uncovered the world of aversive learning in bees and established new research avenues spanning from the evolution of sociality to the molecular bases of aversive learning in insects.

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P 284

Color conditioning of the proboscis extension response in bumblebees

For over a hundred years, classical conditioning has been one of the most intensely studied forms of associative learning. In honeybees, the conditioning of the proboscis extension response (PER) has provided a powerful tool to explore the mechanisms and components underlying olfactory learning and memory. Unfortunately, PER does not work well for visual conditioning of intact honeybees, and only marginally better for antennal-amputated bees, thus limiting the analysis of visual learning and multimodal integration in honeybees. Here, we study visual learning using the PER paradigm in harnessed bumblebees as model system. We have previously shown that Bombus impatiens exhibit high levels of odor learning under restrained conditions. We trained bumblebees in a differential task to associate one color (green, blue or violet) with a reward (A+) while another color was not rewarded (B-). Recording the response latency of the proboscis extension we were able to study two components of the decision-making process: accuracy (as the percentage of correct PERs) and speed (based on latency). We show that bumblebees quickly learn the differential task, reaching more than 50% of conditioned responses after three training trials. However, we observed differences between pairs of colors, with better performance when blue or violet were rewarded. We found a trade-off between accuracy and speed, but we also observed an increase in accuracy and a decrease in latency as learning progressed. Finally, we found a significant association between decision speed and body size, which varied depending on the color rewarded. We conclude that B. impatiens provides a good model to study visual PER conditioning. Bumblebees have proved to be reliable models in electrophysiology, opening new possibilities to analyze the proximate mechanisms of visual learning, as well as the process of multimodal integration and decision-making.

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P 285

Experience-dependent homing ability in honeybees

Landmarks play an important role in insect navigation. For examples, landmarks act as beacons that guide bees and ants to find their path and correct direction; familiar landmarks can retrieve a homing vector that was stored in their memory; bees and ants can also use image matching between the current retinal image with a stored view of the surrounding panorama to find their goal etc. However, how quickly bees learn landmarks along the foraging path and how homing ability depends on experience of foragers are questions that still remain to be answered.

To answer the first question we carried out experiments to investigate 'Relay landscape learning' in honeybees. In the experiment, returning bees from an initial release were collected and then released again at a further distance in the same direction, or in a different direction as a control. The results definitely showed that relay trained bees returned with a significantly higher rate than control bees. This experiment has been done repeatedly in a single-cohort colony and a normal colony. The experiments have been repeated at Michigan State University, USA with the same results.

To answer the second question, we investigated age dependence of homing ability. At different ages, ranging from 8 days to 42 days, bees were displaced by 3000m in the 4 cardinal directions. Results showed that the rate of bees homing successfully improves with age; the return rates from eastern and western directions were also better than those from the other directions. The flight time of the first returning bee and the average flight time of returning bees on the first day improve with the age in the eastern and northern direction. From these two directions, the flight time of the first returned bees with age being older than 3 weeks was ca. 20 min. The flight speed of these bees was at 2.5 m/s. In our experimental region, homing ability does not improved with age in the south and west directions. The reason for this direction specificity is the presence of Black mountain to the west of the hive and Lake Burley-Griffin to the south, which cause bees to rarely forage in these two directions.

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P 286

Modulation of learning by an alarm signal in an insect brain: evidence for an opioid-like pathway.

Cognitive processes can be modulated by many factors, including social signals from conspecifics. In social insects, pheromones are such signals that contribute to organize the life of the colony by modulating the behavior and physiology of individuals. Using the honeybee as a model organism to study the neural bases of learning, we show that appetitive olfactory learning is modulated by a particular social experience, exposure to the sting alarm pheromone. After exposure to this signal (released by other bees in life-threatening situations), bees perform less well in a Pavlovian conditioning assay, compared to unexposed bees. The same result can be obtained after exposure to the main component of the pheromone, isopentyl acetate (IPA). Thus, exposure to IPA may trigger some neuromodulator(s) that modify the function of the brain centers known to be involved in this learning task. Pharmacological treatments combined with the exposure to IPA prior to conditioning show that an agonist (fentanyl) and an antagonist (naloxone) of mammalian opioid receptors can mimick or reverse, respectively, the modulation of learning. This suggests that exposure to the alarm pheromone triggers a signalling pathway that shares common features with the mammalian opioid-system. This hypothesis was already proposed, as IPA exposure also can modulate sensitivity to a noxious stimulus (Núñez et al. 1998). However, the existence of an opioid-like pathway was never clearly demonstrated in Invertebrates. We will present molecular and pharmacological data indicating that such a pathway exists in Insects. Our results provide evidence in favour of functional similarities between at least one or two neuropeptides in the honeybee, and some mammalian opioids. We argue that the action of such neuropeptides may be part of an adaptive physiological response to noxious and alarm/stress signals in the honeybee.

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P 287

Unilateral memory storage in the procerebrum of the terrestrial slug Limax

In most sensory modalities, neuronal inputs are bilaterally processed in a higher center. In some animal species, however, functional lateralization is observed in the sensory processing at the higher level. For the terrestrial slug Limax, olfaction is one of the most important sensory modality and the slug can acquire odor-aversion memories. Previously, it has been demonstrated in bilateral procerebrum (PC) ablation experiments that the PC is necessary for odor-aversion memory, and that the PC is the memory storage site. On the other hand, it has been hypothesized that only the unilateral PC is used for odor-aversion learning. Here we demonstrated that the number of the slugs with normal memory performance was reduced by approximately fifty percent when the PC was surgically ablated only unilaterally before or after conditioning. There was no difference in the memory performance of the right versus the left PC-ablated slugs. However, memory deficit from unilateral PC ablation was not observed when the ipsilateral tentacles were also amputated at the same time. We also showed that there was no lateral memory transfer from one PC to the other, after up to 7 days post- conditioning. Our results demonstrated clearly that either the left or right PC is randomly used for olfactory learning, and that the side of use is determined at the level of the olfactory ascending pathway to the PC.

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P 288

Pattern Discrimination vs. Spatial Learning in Zebra Finches (Taeniopygia guttata)

Zebra finches (Taeniopygia guttata) are able to learn the location of hidden food by orienting on spatial cues in a “dry water maze”, or by discriminating between food feeders with different patterns. At least two distinct brain areas are essential for these abilities: Entopallial lesions cause deficits in pattern discrimination, hippocampal lesions cause strong deficits in spatial orientation. In the present study we tested whether intact birds prefer to learn the position of a baited feeder by spatial orientation or pattern discrimination in a task where both is possible. For this purpose, four food feeders were placed at the floor of a small aviary with extra maze cues. Only one feeder, which was marked with a different pattern, was accessible for food. When the birds had learned to find the food, the location of the differently patterned baited feeder was changed. It was then examined whether the birds preferred the previously learned position in relation to the extra maze cues or the differently patterned food feeder at the new location. The immediate early gene products c-Fos and Zenk were visualized after the test trial to determine the activity of the relevant brain areas. Half of the birds preferred the learned location, the others preferred the differently patterned feeder at a new location. The two remaining positions were not chosen by any of the birds. Expression of the immediate early genes Zenk and c-Fos was enhanced within the hippocampus of birds that preferred location during the test trial compared with those with a pattern preference. Our experiments show that zebra finches are using either the one or the other strategy for orientation. Hippocampal activity during the task correlates with behaviour and suggests that hippocampus is involved in processing of spatial information, but not in pattern discrimination. Supported by the Deutsche Forschungsgemeinschaft (Bi 245/19)

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P 289

Conditioned alarm behavior in piauçu fish (Leporinus macrocephalus)

Piauçu fish like another Ostariophysan fish possesses specialized epidermical club cells that producing alarm substance (AS) that invokes freezing antipredator behavior. Alarm reactions may also occur in response to chemical and visual stimuli that minnows learn to associate with release of alarm pheromone. The current work tested if piauçu can learn to associate a nonbiological, visual stimulus with predation risk. In the first day animals were simultaneously exposed to blue light and distilled water (control, n=6) or AS (n=6). In the second day the animals were rechallenged in the context using blue light only. Light paired with distilled water did not alter ongoing behavior or significantly changes in locomotor (time of locomotion and distance traveled). The result of this control treatment eliminates the possibility that light or injection of a solution result in any behavioral response in piauçu. In contrast, injection of AS provoked freezing behavior characterized by the cessation of all movements as the animal settled to a bottom corner of the aquarium and significantly decrease in the time spent in locomotion ($t=16.126$, $p<0.001$) and distance traveled ($t=6.605$, $p<0.001$). When rechallenged in the context, animals displayed freezing behavior and significantly decrease in the time spent in locomotion ($t=9.425$, $p<0.001$) and distance traveled ($t=15.621$, $p<0.001$). This resulted from a single-trial exposure to the combined cues and demonstrates a robust ecological mechanism by which piauçu fish learn to recognize indicators of predation risk that may vary in space and time. However, learning to associate risk with biologically irrelevant stimuli may be an ecological liability. How piauçu discern between relevant and irrelevant stimuli in nature is not known.

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Study of rat's cardiac activity through a discrimination task

In the present study our aim was to find special patterns of rat's cardiac activity correlated with its behavior during a tactile discrimination task. The approach is based on studies in humans which show a clear correlation between heart activity and conscious intention to perform a motor act (Pfurtscheller et al, 2010) or to take a 'free' decision (Florian et al, 1998; Soon et al., 2008). To this purpose 3 Lister Hooded rats were trained to discriminate between two textures, which are randomly accessible in a central nose-poke. At every trial, after the central touching, rats had to decide between left or right nose-pokes (each side associated with one texture) where they received a reward if the decision was the correct one. During these experiments, we were recording both, behavioral results as reaction-time or percentage of correct and erroneous choices, and the ECG. To carry out ECG records we used 3 skin electrodes place in the animals' thorax. The ECG signal was amplified and digitized by gAmp (g.tec, Austria) and samples were sent to a computer for analysis. Preliminary ECG analysis consisted on QRS complexes detection and calculus of the heart rate (HR) and heart rate variability (HRV) (von Borell et al., 2007). After further analysis involving behavioral and cardiac data, we observed a significant deceleration of the cardiac response after touching the texture. It is in line with several studies in humans which reveal that different kinds of stimulus produce a HR deceleration prior to a response (Druckman and Bjork, 1991). Interesting results have been obtained too after isolating successful experiments (more than 80% correct trials) from unsuccessful ones. It allowed us to study the magnitude of the change in HR related to performance which will present the HR as an indicator of animal's attentive state. Moreover, as HRV has been found as an indicator of stress level (Aubert et al., 1999), the relationship between HRV and decision making will be discussed.

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Mechanosensory systems

586 **P 291**

No pain, no gain: coevolution between bark scorpion pain-inducing toxins and grasshopper mice nociceptors.

Bark scorpions (Centruroides) make toxins that bind Na+ and K+ channels in excitable membranes of vertebrates and invertebrates. The venom produces intense, stinging pain with seizures, paralysis, and respiratory failure. Grasshopper mice (Onychomys) are predators of scorpions. We showed that these mice have evolved resistance to bark scorpion toxins causing seizures, paralysis, and death. Here, we show that they have also evolved resistance to pain-inducing toxins. Electrophysiological analyses demonstrate that peptides in bark scorpion venom target Nav1.7, a Na+ channel expressed in mammalian nociceptors. We tested whether grasshopper mice are insensitive to pain-inducing peptides by injecting samples into their hind paws and measuring the duration of paw licking for 15 minutes; house mice (Mus musculus) were used as a control. Grasshopper mice licked their paws significantly less than house mice, suggesting the former have evolved insensitivity to pain-inducing toxins. To test that other painful stimuli elicit paw licking, we injected formalin into the hind paws of grasshopper mice and house mice. There was no difference between species in the duration of paw licking in response to an injection of formalin, suggesting that insensitivity to pain-inducing neurotoxins is not an inability of grasshopper mice to perceive pain. We cloned and sequenced the gene encoding Nav1.7 from grasshopper mice and compared the sequence with orthologs from other mammals. We identified four amino acid substitutions at highly conserved positions in domain III of grasshopper mice Nav1.7. These results suggest that grasshopper mice have evolved insensitivity to pain-inducing toxins through structural modifications of Nav1.7. We are currently testing whether structural changes to Nav1.7 reduce the effects of the toxin on the channel.

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P 292

The physiological basis of acid insensitivity in the African naked mole rat

Nociceptors are somatic sensory neurons activated by noxious stimuli. Perhaps uniquely among mammals, the African naked mole-rat (NMR, Heterocephalus glaber) displays neither nocifensive behavior to acid nor possesses acid-sensitive C-fiber nociceptors. Nociceptor cell bodies are located in dorsal root ganglia (DRG) and whole-cell patch-clamp was used to measure acid-gated currents of isolated mouse (Mus musculus) and NMR DRG neurons. pH5 evoked sustained inward currents in 85% of mouse nociceptors and transient inward currents in 15% (n=74, peak current density: 19±3 pA/pF). In current-clamp, pH5 evoked APs in only 6% of neurons (n=49). In mouse nociceptors, retrogradely labeled from the skin: transient currents were more frequent (26%), peak current density was greater (40±12 pA/pF, n=27, $p<0.05$) and pH5 evoked APs more often (19%). In NMR DRG neurons, 32% of pH5-gated currents were transient (peak current density: 39±7 pA/pF, n=58) and pH5 evoked APs in 19% of neurons. Although acid causes depolarization, subsequent AP initiation is mediated by voltage-gated sodium channels (NaV) that are inhibited by acid. Total voltage-gated inward currents in mouse DRG neurons were inhibited by acid (IC50 = 5.97) and pH6 inhibited Gmax by 45%. NaV currents mediate the bulk of voltage-gated inward current in DRG neurons and pH6 inhibited isolated NaV current Gmax by 46%. Interestingly, voltage-gated inward currents in NMR DRG neurons are significantly more sensitive to acid: pH6 caused 63% inhibition ($p<0.01$). Greater inhibition may explain the lack of acid-induced nocifensive behavior in NMRs: acid induces inward currents, but increased NaV inhibition compared to mouse prevents AP generation. In conclusion, acid-evoked currents in NMR DRG neurons are similar to currents evoked in mouse skin DRG neurons, but voltage-gated inward currents are more sensitive to acid-inhibition, which may explain the lack of acid-induced nocifensive behavior in NMRs.

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P 293

Chemicals that are often painful do not cause nociceptive responses in crayfish

Whether crustaceans feel pain is often asked, but is complicated to answer. A better delineated question is whether crustaceans have nociceptors: sensory neurons that are specialized to detect tissue damage and stimuli that could damage tissue. Algogenic chemicals bind to nociceptors and cause them to generate action potentials without causing tissue damage. We tested whether crayfish responded to capsaicin (the pungent chemical in chilies and peppers) and isothiocyanate (the pungent chemical in wasabi and mustards). Both chemicals can stimulate nociceptors in vertebrates and invertebrates (although not all species are sensitive to them). Crayfish readily ate foods containing both chemicals (habanero chilies, wasabi rhizomes). We swabbed the antennae of crayfish with solutions containing both chemicals, and saw no differences in behaviour compared to controls in the 10 minutes following application (e.g., no increased grooming, no changes in general movement around a tank, no tailflipping). Extracellular recordings of antennal sensory neurons showed no consistent increase in activity of individual neurons following the application of either capsaicin or isothiocyanate solutions compared to controls. Behavioural and physiological evidence suggests that crustacean nociceptors, if they exist, may be tuned to different stimuli than those in other animals, including other arthropods.

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P 294

A noxious sensory system in developing amphibians: skin impulse access via immature mechanosensory neurons

The skin of some amphibian embryos, like the epithelium of some cnidarians, is electrically excitable. A poke to the skin of Xenopus laevis embryos initiates a cardiac-like impulse that propagates through the skin via gap junctions and can elicit movement by exciting the CNS. The mechanism underlying the connection from non-neural tissue (epithelium) to neural tissue (CNS) remains unclear. In early embryos, access of skin impulse signals to the CNS appears widespread along the brain/spinal cord neuraxis. Behavioural tests using selective lesions show that skin impulse access to the spinal cord becomes progressively less effective with age. By the time of hatching, skin impulse access is only via the Vth (trigeminal) and possibly Ist (olfactory) cranial nerves (Roberts, 1996). Primary sensory Rohon-Beard neurons, which are distributed along the dorsal surface of the spinal cord, innervate the skin with free nerve endings and provide a plausible route for skin impulse access into the spinal cord. However, electrical recordings in immobilised tadpoles showed that individual RB neurons, defined by a clear receptive field in the skin for gentle stroke stimuli, do not respond to skin impulses. In contrast, spikes can be recorded in response to skin impulses in young embryos from probable RB neurons that do not show a clear receptive field. This suggests that skin impulse access to the spinal cord in young embryos is via the immature peripheral neurites of young RB neurons and is lost once they develop mature, mechanoreceptive free nerve endings. Roberts A (1996) Trigeminal pathway for the skin impulse to initiate swimming in hatchling Xenopus embryos. J Physiol 493P, 40-41P.

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P 295

Modulation of spatial processing by somatosensory inputs in the rat

The generation of the spatial cognitive map is influenced by different senses (vision, audition or smell). That the hippocampus also processes tactile information was demonstrated in (Pereira et al., PNAS 104: 18286, 2007). However, the role of somatosensory (tactile) information on spatial representation is unknown. Our hypothesis for this study was that tactile information is relevant for the generation of the spatial map of space. Consequently, the loss of tactile information without the possibility to use cues from other senses (darkness, homogeneous odor, white noise) should affect the spatial representation and could be observed in place cell properties like firing rate, location and/or extension of firing fields. To test this hypothesis we carried out 3 kinds of experiments. First we developed a new paradigm to temporally deprive the tactile input using a local anaesthetic (lidocaine) (Gener et al., J Neurosci Methods, 176: p. 63, 2009). Second, we demonstrated that tactile deprivation by lidocaine worsens tactile discrimination tasks. And third, we applied the deprivation technique to characterise the possible alterations on place cell's representation of space. To assure that the tactile deprivation by local application of lidocaine was effective, we carried out a tactile discrimination task with and without lidocaine application (Lister Hooded, 350grs). Our results were that, after lidocaine, the percentage of successful discriminative responses decayed from 88% to 48%. Once the technique of somatosensory deprivation was developed and validated in the awake animal, it was applied to the characterization of the properties of place cells' firing fields. Our results show that place cells recorded in a controlled environment were sensible to tactile cues, often rotating with the cues rotation. Following deprivation, place cells showed significant changes in the properties of their firing fields. This study suggests that whisker-mediated somatosensory input is a relevant part of the cognitive map creation.

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P 296

Spinal filtering of somatosensory feedback in active sensing

In primates, the corticospinal tract primarily conveys descending motor commands to motoneurons and interneurons in the ventral horn of the spinal cord. However, a significant portion of this tract terminates in the dorsal horn, where it converges with somatosensory input from the dorsal root ganglia. This study tested the hypothesis that this convergence may act to cancel the somatosensory input that predictably results from the descending motor command, thus filtering out the "expected" somatosensory feedback. This filtering would give "unexpected" input ready access to circuitry that could produce corrective reactions. An active sensing task was used, in which human subjects moved the index fingertip across the surface of a virtual sphere created by a robotic device. The fingertip pressed lightly into the sphere during the movement, and the subjects were asked to report the slight differences in sphere size (or surface curvature) which occurred from trial to trial. During each 2-3 second trial, subjects gradually, adaptively adjusted their speed and pressure according to the current surface curvature, achieving a stereotypical level of contact force in the last half of the exploration. Thus subjects were gradually accumulating haptic information about curvature and, at the same time, gradually adjusting the motor commands for the contact force and movement. In humans, a cortically-mediated adjustment to the motor command should take about 100 ms. However, when subjects encountered an unexpected transition in curvature (from circular to flat), they reacted by adaptively decreasing contact force after just 50 ms, i.e., at a spinal reflex latency. Spinal stretch reflexes would have increased, not decreased, force, so this reaction was not simply reflexive, but also appropriately corrective. Therefore, the results show that during active sensing, somatosensory feedback can trigger corrective responses at multiple levels, including the spinal cord.

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Whisking with robots: The computational neuroethology of active vibrissal touch

Computational neuroethology seeks to provide insights into the organization of behaviour by embedding brain models within robotic systems that emulate key aspects of animal physiology. This approach is particularly useful for investigating active sensing since artificial transducers and actuators can place modelled neural circuits in a tight coupling with the real world. The SCRATCHbot (Spatial Cognition and Representation through Active TouCH) robot platform is being developed as an embodied model of the rat whisker system, and to serve as a test-bed for hypotheses about whisking pattern generation and vibrissal signal processing. To provide an effective model we emulate key physical elements of the rat whisker system at a scale approximately 4x larger. The robot â€˜snoutâ€™ supports left and right 3x3 arrays of macro-vibrissae, with each vibrissal column actuated using a separate, miniature motor. An array of short, non-actuated whiskers at the snout tip emulates the micro-vibrissae. Joints in the neck provide pitch, yaw and elevation control, whilst the body is supported and moved by three independent motor drive units. This configuration provides similar degrees of freedom for head and whiskers positioning as are available to the rat. The vibrissal shafts are made from a synthetic polymer with shape and material properties similar to those of rat whiskers; each whisker base is mounted in a Hall-effect transducer that accurately measures deflection in two directions. The robot control system incorporates models of sensorimotor loops at multiple levels of the neuraxis. The behaviours we currently emulate include anticipatory and feedback control of bilateral whisking pattern generation, and an orienting response mediated by a model superior colliculus. Data obtained by exploring target surfaces with the vibrissae is analyzed using biologically-inspired algorithms for texture and shape recognition. This research is support by the Framework 7 BIOTACT project.

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P 298

Active touch in the rodent vibrissal system: the development of whisker control in neonatal rats

Animals actively regulate the position and movement of their sensory systems in order to boost the quality and quantity of the sensory information they obtain. The rat vibrissal system is recognized to be an important model system in which to investigate such â€œactive sensingâ€? capabilities. Adult rats sweep their large facial whiskers (macrovibrissae) back and forth in a rhythmic behavior known as whisking that is observed under many conditions of natural locomotion and exploration. Recent research in our laboratory has demonstrated that exploratory whisking, in freely-moving animals, often diverges from the regular, bilaterally-symmetric and synchronous motor pattern that has been recorded when immobilized rats are trained to whisk in air. Specifically, we have documented asymmetries, asynchronies, and changes in whisk amplitude, timing, and in whisker angular separation (spread), that may be the consequence of â€œactive sensingâ€? control strategies (Mitchinson et al., Proc. Biol. Sci. 274:1035-41, 2007; Grant et al., J. Neurophys. 101:862-874, 2008). In the current study we describe the development of these whisker control strategies in rat pups between post-natal day (P) 1 to 21. We analysed 966 short video clips of thirteen pups from six litters and measured the emergence of whisker control behaviours alongside the emergence of locomotion. Prior to P10, whisker movements are limited to unilateral retractions accompanying head turning. Between P10 to P15 bilateral whisking emerges. Contact-induced bilateral asymmetry and asynchrony, and control of whisker spread, all appear post-P14. Comparison of these results with known data on the maturation of key brain structures suggests that these active control strategies depend upon, or are moderated by, structures in the rat midbrain and neocortex including the superior colliculus and the somatosensory cortex, and that their emergence may be experience-dependant.

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The nematode touch response facilitates escape from predacious fungi

Gentle touch to anterior half of the body of free-living nematodes induces an escape response in which the animal reverses and suppresses exploratory head movements. We have analyzed the neural circuit of the escape response of the nematode C. elegans and found that the trace amine, tyramine, plays a crucial role in the escape response. Tyramine coordinates the backing response and the suppression of head movements through the activation of the tyramine-gated chloride channel, LGC-55. In the soil the main predators of nematodes are predacious fungi, which catch and devour nematodes using hyphal trapping devices. The most sophisticated nematophagous fungi form ring shaped traps that swell rapidly when stimulated by the touch of a nematode. We hypothesized that the touch-induced suppression of head movements could allow the animal to smoothly retreat from the constricting ring and evade capture. We therefore examined predator-prey relations between the fungus Monacrosporium doedycoides and C. elegans. Early larval stages of C. elegans can pass through the fungal rings and were caught most efficiently whereas adults are too large to crawl through the traps and were caught less frequently. There is a delay of approximately 5 seconds between the initial entry into the ring and trap closure, which allows the animal to withdraw from the trap before being caught. Wild-type animals managed to escape from a trap in 80% of the encounters, whereas touch-insensitive mec-4 mutants only managed to escape in 44% of the encounters. Tyramine deficient (tdc-1) or lgc-55 mutants, that sense touch but fail to suppress head movements, also escaped less efficiently from traps (50-60%, respectively). In competition experiments with mixed populations of wild-type and lgc-55 mutant animals, we found that the lgc-55 mutant animals were caught significantly more frequently. We therefore conclude that the suppression of head oscillations is an ecologically relevant behavior that allows the animal to smoothly retract from a deadly fungal noose fast enough to evade capture.

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Revealing the Neural Controller in Antenna-Mediated Tactile Navigation in Cockroaches

The integration of sensory stimuli for high-bandwidth task-level control is a fundamental challenge for locomoting organisms, particularly when sensory delays are significant. Cockroaches rely on mechanosensory structures in their antennae to tactilely track surfaces during high-speed running. Control theoretic models of neuroethological behaviors such as wall-following in cockroaches provide testable hypotheses for neural encoding. A simple control theoretic model suggests a strategy relying on proportional and derivative information of wall distance that is sufficient for both simple template and more dynamically representative models of wall following. Results from template and anchored models provide a framework for generating hypotheses of sensory encoding. Extracellular whole-nerve recordings of mechanoreceptive afferents in the flagellum of the cockroach antenna are consistent with this model showing phasic and tonic components in the envelope neural response. We hypothesized that individual mechanoreceptive units explicitly encode velocity and position signals during behaviorally-relevant antenna displacements. We recorded and identified single mechanoreceptive units in fixed neurophysiological preparations from the antennal nerve with en passant suction recording in the cockroach Periplaneta americana. The antenna flagellum was driven with a voice-coil actuator to evoke virtual turning stimuli in antenna position. Recordings reveal velocity dependent firing rates but no detectable position dependence. A position signal may be generated by a distribution of threshold-sensitive receptors along the flagellum which we may not have detected at the single unit level. Alternatively a position signal may be generated at the population level by delay-line processing. In addition to generating hypotheses for sensory encoding, studying cockroach wall-following within a control theoretic framework has led to the development of novel bio-inspired systems.

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P 301

Evolution of propriosensory organs on the legs of flies

Many arthropods have proprioceptive organs consisting of small groups of innervated mechanosensory hairs on the first segment of the leg, the coxa, that are differentially deflected by various positions of the coxa relative to the thorax and thus the hairs monitor leg posture. The legs of insects are homologous, but it is an open question to what extent the sense organ at the base of the legs is modified among insect groups and across the three legs within a species. In flies (Diptera), we have examined the arrangement and position of hairs with light and scanning electron microscopy, and the axonal projection into the ganglia with confocal fluorescent microscopy following dye filling of the hairs. Of specimens examined thus far, each coxa has an anterior and a posterior hair plate. The number and arrangement of the hairs, however, varies across the ca. 125 families of flies. More primitive flies have a loose cluster of hairs numbering up to 20 or so. In the phylogeny of flies, around the level of the suborder Cyclorrhapha the pattern of hairs becomes fixed: two rows of 4 hairs for the anterior hair plate and a single row of 4 hairs in the posterior plate. Even among flies in which the number of hairs has become fixed, there are still modifications of placement around the coxa depending upon which leg the hairs monitor. In Sarcophaga bullata (Sarcophagidae) the three coxae are more or less restricted in angular movement and the position of the hairs varies across the legs depending upon the amount and direction of movement. In contrast to the variable arrangement of sensory hairs, the axonal projections appear stereotyped across the flies and their close relatives, the scorpionflies (Mecoptera). Indeed, sensory projections from a fly hair plate look very similar to those of a cockroach. These results demonstrate that external morphology of homologous sense organs is much more varied and may be more responsive to evolutionarily modification, whereas internal sensory projection patterns are more conserved and may be more constrained.

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Functional dissection of filiform sensilla in the bug Pyrrhocoris apterus

Mechanoreceptors are a group of sensory receptors that detect stimuli such as touch, air current, sound, etc. The unifying paradigm connecting this diverse group of receptors is direct transmission of the stimulus force on the transduction channels. In recent years important advancements have been made in the study of their molecular machinery. However, due to their unapproachability to direct electrophysiological measurements, many basic principles of their function remain unclear. In this poster we present the analysis of the resting activity and adaptation processes of filiform sensilla in the bug Pyrrhocoris apterus. Filiform sensilla are simple receptors consisting of a single sensory cell. In P. apterus they are classified into three types, T1, T2, T3, according to their structural and functional properties. The goal of our study is to dissect the functional properties of intact sensilla by computational methods. In the first part of the poster we introduce a novel method, which allows to reconstruct the time course of the membrane potential between two subsequent spikes from extracellularly recorded spikes. With this method we show that at rest T1 is operating in a superthreshold regime, resulting in a regular, high rate spontaneous activity. In contrast, T2 is operating in a subthreshold regime, resulting in a more irregular, low rate activity. In the second part we attempt to disentangle two potential adaptation processes of T1 sensilla with similar temporal dynamics. The first process takes place on the level of the stimulus transmission and transduction, which we model as a damped oscillator. In the second process the generated action potentials activate slow adaptation currents. For the latter we obtain the adaptation time constant from serial correlations of interspike intervals during resting activity. With these two processes we successfully reproduce the basic features of the response of the sensilla to various stimuli.

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Caterpillar crawling over irregular terrain: anticipation and local sensing

Animals moving in complex environments must identify objects in their path and know the relative position of the object to their limbs. For soft bodied locomotion this is a particularly difficult problem because tissues can deform and proprioceptive information may not predict body position. In the absence of remote sensing such as vision or sonar, objects must be detected by touch. Object avoidance could be achieved by gathering mechanosensory information in leading parts of the body and then using it to adjust movements in the following limbs. *Manduca sexta* caterpillars can negotiate small objects in their path by stepping on top of them. During such a step, the maximum upward velocity of the approaching prolegs in the first quartile of the swing phase was much higher than that of a normal step, even when the prolegs were located far from the obstacle. This increased lift rate occurs before the prolegs touch the obstacle indicating that sensory information from more anterior body segments is being used to alter its trajectory: *Manduca* uses intersegmental information to anticipate objects. As the caterpillar's soft body deforms due to locomotion between receiving the sensory information and encountering the object with the prolegs, proprioreceptive information is likely to be used for an appropriate adjustment of the legs' trajectory. When stepping on the obstacle the caterpillar shortens its prolegs more than in a normal step so the planta retractor muscles are good candidates for receiving neural input about the upcoming obstacle. This neural input is likely to originate from sensory hairs on the ventral surface of the two legless body segments anterior to the prolegs. The neural communication from these sensory hairs to the retractor muscles in more posterior body segments are being investigated using electrophysiology techniques.

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613 **P 304**

Neurobiology of Drosophila gravitaxis behaviour

It is well established that the behavior of the fruit fly, Drosophila melanogaster, is affected by gravity. One example of such behavior is the negative gravitaxis flies show when shaken to the bottom of a vial or while traversing a Hirsch maze. Although the simplicity of these assays has made them ideal for genetic screens seeking the molecular basis of gravitactic behavior, this approach has yielded only a coarse understanding of the contributions of different sensory structures and even less about subsequent processing. The halteres, neck bristles, leg and wing proprioceptors, and the antennal Johnston's organ (JO) have all been functionally implicated in graviception. The most recent studies describe a functional role for subsets of JO neurons and their projections. We set out to contribute to this existing knowledge by establishing behavioral assays that allow the analysis of individual flies at the relevant behavioral timescales of seconds to minutes. The developed assay exposes groups of flies (20-30) to static orientations in reference to the gravity vector, while videos are acquired for off-line analysis. Our approach allows the analysis of detailed gravity-response measurements such as minimum detection threshold, reaction times, and the optimal response level. In addition, we are using the assay to conduct a moderate-size screen through available Gal4-lines enabling functional manipulation of small numbers of neurons. By selecting Gal4-lines that should, by neuroanatomical inference, affect the halteres, the antenna, or the central complex, we aim to specifically analyze the contribution of different sensory structures and brain regions.

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P 305

Temporal processing of species-specific vibratory signals at the level of ascending interneurons in Nezara viridula (Hemiptera: Pentatomidae)

Insect vibratory communication signals differ substantially from their airborne sounds, often having lower frequencies and pure tones, while the sensory system designed for receiving these signals involves numerous inputs from all six legs and the antennae. This is reflected in a complexity at the level of the ascending neurons (Zorovic et al, 2008) that far surpasses the relatively simple auditory system of crickets, one of the most commonly investigated models for studying insect sound communication. However, for them, as well as the vibrationally communicating bugs like N. viridula (Hemiptera), the recognition of the species-specific temporal pattern of the communication signals is vital for successful pair formation. It was previously assumed that in the cricket auditory system, temporal filtering only takes place in the brain; however, Nabatiyan et al. (2003) showed that some temporal filtering, based on the instantaneous spike rate, occurs at an early stage of the auditory pathway at the level of local prothoracic interneurons. We investigated whether, in the more complex vibratory signal pathway in N. viridula, temporal filtering also occurs at the early levels of processing, before the signal reaches the brain. In the experimental set-up, the stimuli were delivered to all six legs via a minishaker while the activity of the ascending and local neurons was recorded intracellularly from the central ganglion. The test sequences included vibratory songs with various combinations of pulse and interval duration. We show that temporal filtering is indeed present already at the level of the central ganglion. We describe several morphological types of local and ascending vibratory interneurons that exhibit either low pass or band pass filtering for the pulse duration based on the number of spikes as well as the maximum spike rates, but no filtering for the interval duration.

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P 306

Vibrational signalling in the non-hearing cave cricket and corresponding responses of neurons in the ventral nerve chord and the brain

Cave crickets (Rhaphidophoridae) represent an under investigated group of Ensifera with respect to mating behaviour and communication, since they neither hear nor emit sound. In this study we describe the complete process of courtship and mating together with the substrate-borne vibrational signalling in the mid-European cave cricket Troglophilus neglectus. Males produce substrate vibration signals with abdominal oscillations during the close range courtship. As detected by laser vibrometry, only one type of signals is produced with the mean duration of 566 ms and repetition time of 2.2 s. Most of the signals' spectral energy lies below 300 Hz with the dominant frequency between 80-90 Hz. At the point of measurements, 5−10 cm away from the signaller, the peak velocity of signals ranged between 2.5*10-5 − 10-6 m/s. In the prothoracic nerve chord ganglion of T. neglectus six most highly sensitive vibratory interneurons that were previously identified respond to the respective range of frequencies and intensities; only one neuron, however, conveys this information directly towards the brain. To determine to what degree the vibrational system of T. neglectus may be adapted to detect intraspecific signals at further processing levels, we investigated spectral sensitivity and responses to play-backed male signals in the brain neurons using intracellular recording. So far two (groups of) local neurons have been identified in the lateral protocerebrum, with broadband excitatory "on" and "off" responses and inhibitory responses to vibration, respectively. In addition, three different types of physiological responses have been recorded, expressing low-, middle- and high-frequency sensitivity, respectively. Of these neurons, only one low-frequency tuned element responded to the signals of the male at the relevant intensity.

Stritih, Nata¹a

Dr.

706 **P 307**

Vibration signal processing by tarsal slit sensilla of sand scorpions

As animals move over dry sand, slippage of surface grains within the Earth's gravitational field generate vibrational signals in episodic bursts (i.e., milliseconds duration) of highly variable amplitude (0.001 to 10 m/sec2 acceleration) and broad, frequency-attenuated bandwidth (300->5000 Hz). The surface acoustical waves propagating away from these disturbances can trigger accurate predatory pursuit behavior in the nocturnal sand scorpion, Smerigurus (formerly Paruroctonus) mesaensis. Eight specialized vibration receptors, the basitarsal slit sensilla, at the tips of the scorpion's outstretched legs sense these passing waves and process the signal timing and amplitude information used to compute location of the moving prey. Here we quantify the signal characteristics of natural microseismic signals in dry sand and correlate the sensory response of slit organs to this broadband vibrational input (threshold ~10-10 m amplitude). Each slit within the compound organ responds with a distinct, fatigue-resistant spike that is weakly phase-locked to sustained oscillation over the 500-2000 Hz bandwidth that dominates the sand vibrational signal. Synaptic interactions between sensory cells of the compound organ, or originating from CNS efferent feedback, act to modulate the sensory response over the wide range (50 dB) of signal intensities encountered. Such modulation may protect the organ from desensitization or synchronize the integrated response from all slits. The properties of this vibrational sensory system in sand scorpions show characteristics similar to auditory sound localizing systems of other animals suggesting convergence of the physiological mechanisms that extract amplitude and timing information over a wide range of signal intensities.

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P 308

NEURONAL ENCODING OF WIND GENERATED BY BATS ATTACKING THE BUSH CRICKET TETTIGONIA CANTANS

For many insects bats constitute a major predation pressure. As an adaptation to bat predation the auditory system of many insects is sensitive to echolocation calls emitted by bats. Ultrasound sensitivity allows insects the performance of an escape maneuver following the detection of an approaching bat. Recently, the European mouse-eared bat species Myotis myotis was found to eavesdrop on the calling songs of Tettigonia cantans (Jones et al. 2010). Due to the high syllable rate (~37 Hz) of this insect, the detection of rather faint echolocation calls emitted by attacking gleaning bats may be difficult. In this study we investigate the possibility whether wind-sensitive hairs on the cercal organ of T. cantans may forewarn singing males about attacking bats. The activity of giant interneurons sensitive to wind was recorded by means of a hook electrode during real attacks of the European mouse-eared bat species Myotis myotis. These experiments were conducted in a flight room at the Max Planck Institute in Seewiesen, Germany. Flight tracks of bats were monitored using 3 simultaneously operating infrared video cameras. Approaching bats generated a maximum wind velocity of 0.58 ± 0.14 m/s at the position of the cercal organs. Three different units of dorsal giant interneurons responded to attacking bats with firing regular bursts. Between the first neuronal response and contact, a mean time lag of 860 ms was found. This should give insects enough time for the performance of a last chance evasive manoeuvre. This result suggests the cercal system of Orthopterans as an effective system for the ready detection of attacking bats and corroborates results obtained with praying mantis (Triblehorn and Yager 2006). Thus, the cercal organ may be life-saving for singing insects suffering from eavesdropping by bats. Jones, P., Page, R. Hartbauer, M. and Siemers, B. (2010) Behav. Ecol. Soc. in press Triblehorn, J. D. and Yager, D. D. (2006): J. Exp. Biol. 209, 1430-1440.

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673 **P 309**

Cercal Wind-Sensing System of Crickets: Investigating the Sensory Neurons.

Crickets, like other insect taxa, possess a pair of posterior sense organs, the cerci, which bear sensory hairs for the detection of air flow. These so-called filiform hairs consist of a long thin shaft viscously coupled to the motion of the surrounding air. Hair deflections are detected by a mechanosensory neuron, which relays the resulting spike trains to the terminal ganglion of the central nervous system. It has been demonstrated in behavioral studies that the wind-elicited escape response of crickets can be fast and reliable. Special attention has been paid to the neural mechanism of the evaluation of stimulus direction by the ascending first-order interneurons, which are believed to control the escape response. Some basic questions, however, still remain open. What features of a stimulus can be resolved and at which accuracy? To derive hypotheses for the function and capabilities of the system it is necessary to know the performance of a single sensor. We have therefore designed a setup to record the afferent responses of a filiform-hair sensory neuron of Gryllus bimaculatus. The setup consisted of a moving box producing the air flow stimulus with the cricket placed inside. We have measured both the filiform-hair activity by means of an extracellular electrode and the hair motion in 3D simultaneously through two high-speed cameras. This has allowed us to correlate air motion, hair motion, and sensor activity. First analyses show that inter-spike intervals were distributed differently in different sensory neurons. Furthermore, inter-spike intervals appear to be a rather complex function of stimulus velocity and acceleration as well as spike history, i.e., preceding spike intervals.

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P 310

Function from Form: Projective Field Analysis in the Cricket

The cricket cercal system has been used as a model sensory system for several decades. This system detects near-field air motion using hundreds of unidirectional mechanosensory hairs. Primary afferents from these hairs synapse onto 20 projecting giant interneurons (INs). The response of many of these INs to the direction of bulk airflow is well understood and has been used as a textbook model of synergistic coding. However, recent work in our lab has shown that these INs are highly sensitive to both the location of a stimulus along the cercal array as well as to the timing of stimulus arrival at these various locations. The textbook understanding of these INs considers only the stimulus subspace where stimuli excite the entire cercal array simultaneously. The complete stimulus space for these cells is far more complex both spatially and temporally. The assumptions behind previously used white-noise exploration of stimulus spaces have been shown to be problematic both theoretically and practically for such a large stimulus space. We wished instead to use a projective field approach based on an IN's morphology and presynaptic partners. Highly accurate three-dimensional computer models of these neuron's passive dendritic trees and their probabilistic presynaptic afferent partners have been created in previous studies. Putting this information together allowed us to create accurate computational models of the complete passive feed-forward pathway from stimulus to IN firing. We then used these models to predict IN tuning in the appropriate temporospatial stimulus space. These model predictions were then compared to in vivo recordings of the same INs. A novel recording method allows us to simultaneously obtain and sort spike trains from all 20 ascending interneurons of the system. This provides a useful test not only of our neuronal models, but also of the utility of a projective field approach in determining a neuron's multidimensional tuning curve. By considering only the excitatory feed-forward signal our models do a good job of predicting IN responsiveness. We were additionally able to quantify this 'goodness', which provides a measurement of the significance of mechanisms and factors not considered by the model. We have thus obtained a quantitative assessment of how well simple anatomical data can predict neuronal behavior.

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Regulation of axonal conduction velocity from near-field receptors on the crayfish antennule.

The antennular flagella of the crayfish Procambarus clarkii each possesses a linear array of near-field receptors, termed standing feathered sensilla, that are extremely sensitive to movement of the surrounding water. Previously it had been shown that, within each flagellum, the axonal conduction velocity of the sensory neuron pair associated with each feathered sensillum was linearly related to the position of the sensillum along the flagellar axis. In the current studies I show that the conduction velocity of axons within the proximal three segments of the antennules, between the flagella and the brain, is similar in most axons examined, regardless of their point of origin on the flagellum, with an average conduction velocity that was higher than the mean conduction velocity of even the fastest axons within the flagellum. One consequence of this change in axonal conduction properties is an effective compression of the temporal spread - potentially by as much as ten fold - which otherwise would occur in arrival times of initial spikes from each sensillum following a mechanical stimulus to the antennule. Furthermore, the pattern signature of initial spike volleys at the brain following a global hydrodynamic stimulus to the flagellum is remarkably consistent and conceivably could be recognized as such by central processing centers. I conclude that conduction velocity adjustments improve temporal summation and resolution from input volleys that originate in the highly sensitive and, hence, inherently noisy near-field receptors, thereby more effectively triggering startle response circuitry at the approach of potential predators.

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P 312

Antennal Positioning in flying insects

During flight, many insects maintain their antennae in a fixed position relative to the head. Since antennal mechanosensory input is required for flight control (Sane et al 2007), this "antennal positioning behaviour" is likely to be important during flight. We show using diverse techniques, that antennal positioning in the hawkmoth Daphnis nerii is mediated by antennal mechanosensory organs called the Bohm"s bristles. These bristles are organized in fields on the scape and pedicel of the antenna. We measured how antennal positioning is influenced when the mechanosensory inputs are reduced. Reducing input from Johnston’s organs by restricting the pedicel-flagellar joint does not alter antennal positioning, and neither does ablation of the pedicellar Bohm’s bristles. In contrast, ablation of the scapal bristles causes severe mispositioning of the antenna, increasing the incidence of wing-antennal collisions. Thus, scapal bristles are primarily responsible for antennal positioning. We proceeded to visualize the neural circuitry underlying the Bohm’s bristles using fluorescent dextran dyes and confocal microscopy. Sensory neurons innervating the Bohm’s bristles arborise in a region known as the Antennal Motor and Mechanosensory Centre where they overlap with the dendritic arbor of antennal motor neurons. Electromyographic recordings from the antennal musculature show that the muscles respond to stimulation of the bristles, confirming that the two are connected. This connection may be monosynaptic, making this sensorimotor circuit a classical reflex arc. We propose that antennal positioning can be described as a negative feedback loop in which deviations from the antennal position are detected by the Bohm’s bristles and translated into corrective responses of the antennal muscles to maintain the antennal position. Since knowledge of antennal position is critical for several behaviours e.g. tactile responses, etc. this mechanism is likely conserved across insect orders.

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P 313

Mechanical coupling of the wings and halteres in the black soldier fly, Hermetia illucens

Insects perform exquisite maneuvers during flight. In Diptera, these maneuvers require precise integration of visual feedback from the compound eyes and mechanosensory feedback from the primary balancing organs called the halteres, dumb-bell shaped sensory organs which evolved from the hind wings. Together, these sensory modalities inform the central nervous system about the insect's flight status and drive the fine adjustments in wing kinematics essential for flight control. Halteres act as vibrational gyroscopes and provide information about insect's angular velocities along the roll, yaw and pitch axes (Pringle, 1948). Like the wings from which they are derived, halteres also oscillate during flight at wing beat frequencies, but their vibrations occur in a strict anti-phase relationship to the wing motion. Because the phase of haltere sensory input with respect to the wing mechanosensory input modulates activity of wing steering muscles (Fayyazuddin and Dickinson, 1999), it is likely that the precise anti-phase coordination between wings and halteres is essential for equilibrium during flight. What mechanisms drive the coordination between wings and halteres? Here, we show that wing-wing and wing-haltere coordination result from mechanical coupling. Through a series of experiments on freshly dead soldier flies, we show that wing-wing and wing-haltere maintain their strict in-phase and anti-phase movement respectively in spite of complete absence of neural activity. These experiments reveal that the coupling element for this coordination, but not ipsilateral wing-haltere coordination, lies in the scutellum of the insect thorax. Our experiments suggest that the basic coordination of wings and halteres is inherent within the physical structure of the thorax. Sensorimotor feedback may provide a modulatory influence to this system during active maneuvers.

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Predicting haltere neuron spike trains in freely-flying dipterans

The halteres of dipteran insects are reduced hindwings that possess fast, accurate cuticular mechanoreceptors at their bases. These mechanoreceptors detect strains that deform the cuticle during body rotations and send information about these strains to the central nervous system. While these small strains are not measurable in vivo during natural flight, they can be measured using computational finite element methods. We constructed a finite element model of the haltere of a crane fly (Holorusia hespera) based on anatomical and biomechanical measurements of halteres. We filmed the halteres of freely-flying crane flies with high-speed digital video, and then actuated the finite element model so that its tip motions were similar to the tip motions seen during free flight. In doing so, we were able to approximate the strain that occurs at the location of haltere sensory cells during natural behavior. This strain information was then combined with current models of haltere neuron feature detection to predict trains of spikes in the haltere primary afferent neurons. By predicting the spike activity of several neurons in the haltere, we are able to better understand how the haltere system transmits information about forces on the body to both the wing-steering motor system and to the brain.

Fox, Jessica

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Dynamic encoding of wing bending by mechanoreceptors in the hawkmoth Manduca sexta

The wings of all insects have strain-sensitive campaniform sensilla. In large insects, such as Manduca sexta, the wing surfaces deform significantly during flight. Moreover, the degree to which wings deform is an important determinant of the magnitude of the aerodynamic forces that they generate. Thus, information about wing bending could have an important behavioral role as these animals move about their environment. The extent to which the nervous system can encode such information, however, remains unknown. To address this issue, we present a band-limited Gaussian white noise mechanical stimulus to the wings of moths while simultaneously recording intracellularly from the primary afferents that innervate the campaniform sensilla on the wing. We use signal processing and information theoretic analyses to assess temporal precision, frequency tuning, and coding capacity in these cells. We find that the temporal uncertainty in arrival times of spikes in response to repeated presentations of a stimulus (jitter) is between 0.2 and 3 ms, up to two orders of magnitude smaller than the wing stroke duration of 40 ms, and that the cells are maximally sensitive to frequencies of about 150 Hz, much higher than the natural wing beat frequency (25 Hz). Additionally, we find linear estimates of information transmission rates in excess of 130 bits/second and 3 bits/spike, commensurate with some of the highest information transfer rates that have been reported for sensory systems in the literature. Our results suggest that these mechanosensory neurons can encode and transmit strain information at rates that are sufficiently fast to detect bending waves in flapping wings. Moreover, they have the capability of reporting inertial forces associated with wing flapping.

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Heterogeneity and function of posterior lateral line afferent neurons in larval zebrafish

Afferent neurons of the zebrafish posterior lateral line relay hydrodynamic information sensed along the body to the hindbrain. I electroporated individual neurons with Alexa 647 in HUC-Kaede fish, a transgenic line expressing a photo-convertible protein under control of a pan-neuronal promotor, to reveal that single and multiple-neuromast afferent neurons correspond to later and early-born cells, respectively. I normalized ganglion area across individuals and plotted position of afferent neurons to show that early-born cells are located centrally in the ganglion, with newly developing cells added to the periphery. Whole-cell recordings of afferent neurons show an inverse relationship between soma area and input resistance, where input resistance is a proxy for excitability. In vivo patch clamp recordings from afferent neurons identified two classes of cells based on their spontaneous activity and intrinsic firing properties; tonically firing and phasically firing. Tonically firing cells fire single spikes at a frequency of 57.2 ± 5.3 Hz (mean ± standard error, n= 4 fish) in the absence of hydrodynamic stimuli. Their firing frequency increased upon depolarizing steps (20-80 pA, 500 ms duration). When a jet of water is introduced along the body, multiple, high frequency spike bursts (214.9 ± 25.8 Hz, mean ± standard error, n= 4 fish) are superimposed onto the tonic background of single spikes. Phasically firing neurons are silent at rest, exhibit several spikes only at the beginning of a depolarizing step (20-80 pA, 500 ms duration), and do not fire consistently to hydrodynamic stimuli. Taken together, a picture is emerging that large, early-born cells are less excitable and may therefore fire only to strong hydrodynamic stimuli across the whole body, while small, later-born cells that are more excitable sense local flows. Therefore, large, coarse coding afferents innervating multiple hair cells may be critical for initiating powerful escape responses while small, fine coding afferents may be reserved for modulating routine motor behaviors such as swimming.

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P 317

Gentamicin disrupts both receptor classes in the lateral line system.

Many behaviors exhibited by aquatic animals rely on the ability to sense water flow. In fish, flow sensation is mediated by hair cells within the lateral line system. This system is composed of two classes of receptors: superficial and canal neuromasts. Ethological investigations have sought to separate the roles of these two receptor classes using an aminoglycoside antibiotic, gentamicin. Gentamicin is believed to disrupt the function of canal, but not superficial, neuromasts. We tested this theory in vivo. In this study we used florescent vital dyes (DASPEI and FM1-43) following exposure to a solution of gentamicin. Contrary to the prevailing assumptions, we found that gentamicin disrupts the hair cells in both receptor classes. A significant effect was found for both the superficial and canal neuromasts of two different fish species (*Astyanax mexicanus* and *Danio rerio*). Furthermore, by labeling hair cells prior to gentamicin exposure, we observed that, in both classes, disrupted hair cell function is at least partially due to cell death. We conclude that gentamicin is not a reliably selective blocker of canal neuromasts. In light of this result, we have revisited the effect of gentamicin exposure on rheotaxis, an unconditioned orienting response to water flow. Prior studies have concluded that gentamicin exposure does not affect the rheotactic response, and that canal neuromasts are not important for rheotaxis. However, after carefully validating drug exposure with vital dye staining, we found that gentamicin exposure disrupted rheotaxis in the blind Mexican cave fish, *Astyanax mexicanus*. These results demand re-evaluation of many prior behavioral studies of the lateral line system.

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Predation pressure drives lateral line variability in guppies

The ability to detect and escape potential predators is essential to an organismâ€™s survival and fitness. As a result, predation pressures frequently drive evolution, leading to phenotypic change and adaptation. The mechanosensory lateral line enables fish to detect objects and motion in their immediate environment. Variation in lateral line morphology allows different fish to more effectively detect and respond to salient cues, including predators. We hypothesized that differences in predation pressure in different populations of the Trinidadian guppy (Poecilia reticulata) might reflect differences in lateral line morphology. We sampled a high and low predation population from the Guanapo river drainage in Trinidad and examined patterns of neuromast distribution across the body. Although there was distinct overlap in neuromast distribution in the belly of fish of the two populations, high predation individuals had more variable distribution, on dorsal and lateral areas as compared to their low predation counterparts. These differences reflect differential water column use between the two populations. High predation guppies tend to remain just below the waterâ€™s surface to avoid predators from below, while low predations guppies move throughout the water column. Our data demonstrate that the lateral line sensory system can vary between populations, and suggest that these differences may be driven by differences in predation pressure. Future work aims to understand how much of the variation between populations is genetically based and how much is a plastic response to environmental differences.

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P 319

Discrimination of complex hydrodynamic stimuli in rheophilic fish

Fish perceive local water movements and pressure gradients with their mechanosensory lateral line. Fish use hydrodynamic information for orientation, prey detection, predator avoidance, schooling and for inner-specific communication. The lateral line of fish may also be essential for the detection and discrimination of vortex streets, caused, for instance, by moving conspecifics or by stationary objects exposed to running water. Rheophilic fish frequent Kármán vortex streets to minimize their energy expenditure during station holding. Whether these fish actually can sense and discriminate vortex streets is not known. We investigated whether roach (Rutilus rutilus) can perceive Kármán vortex streets. In a two-alternative forced-choice task we trained roach to enter the compartment of a flow tank in which a vortex street was generated. To exclude visual cues all experiments were conducted under infrared illumination. In further experiments we will test whether roach not only can detect but also can discriminate vortex streets that differ in either vortex shedding frequency and/or in vortex turning directions.

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P 320

PRESSURE AND TEMPERATURE RELATED NON-LINEARITIES IN THE SENSING OF ANGULAR ACCELERATION IN SHORE CRABS

The crab balancing organ is used during navigation both for the sensing of angular acceleration as well as changes in depth. Pressure sensing is believed to occur through the differential compression of fluid within the thread hair acting on a piston-like chorda. This piston model was tested on its predictions that the effects of temperature on the volume of fluid should counter- act the effects of pressure. By first exposing thread hairs to sinusoidal pressure cycles of 0.15- 0.36 bar and then adding temperature cycles of 13-24ºC, this study aimed to examine the combined effects of pressure and temperature on the frequency of thread hair signalling in response to rotation. Clear bursts in elevated spike frequency occurred during simultaneous changes to pressure and temperature, consistent with the piston model. Measurements on averaged extracellular spikes also suggested changes in spike characteristics including peaks and rates of rise during these bursts which may be indicative of a changed pattern of recruitment of sensory spikes or of changes in the electrical characteristics of the spikes. These findings together with the occurrence of unexplained transients when testing the nerve with temperature change alone, also raise the question of the effects of temperature and pressure on the sensory nerve lipid membrane and its influence on the sensory signal and action potential transmission.

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P 321

How depth reception can affect the distributions of marine organisms

Depth reception is known to be a property of angular acceleration receptors in the vestibular systems of crabs and sharks, and clearly is found in a variety of other marine animals. Resting activity is known to be modulated by small changes in hydrostatic pressure which is a natural proxy for depth. Such modulation follows at up to tidal periods greater than 12 hours, with two directional classes of units known in crabs. The hydrostatic pressure sensing system hence is one of the most tonic, non adapting sensory systems known. Depth reception is now thought to underlie a variety of significant processes involved in the orientation and locomotion of marine animals ranging from the synchronizing of tidal rhythms to the spectacular compensatory swimming maintaining station in vertical currents which has been shown to account for the horizontal distributions of plankton in inshore bays. The mechanism involves differential compression and activation of mechanoreceptors and interesting transients may be explained by the involvement of lipids at their transition point. • Fraser, P.J. and A.G. Macdonald (1994) Crab hydrostatic pressure sensors. Nature 371, 383-384 • Fraser , P.J. and R.L. Shelmerdine (2002) Fish physiology: Dogfish hair cells sense hydrostatic pressure. Nature 415, 495-496) • Fraser, P.J., S.F, Cruickshank, R.L. Shelmerdine and L.E. Smith (2008) Hydrostatic pressure receptors and depth usage in crustacea and fish. Navigation: Journal of the Institute of Navigation Special Issue on Bio-Navigation 55, No. 2, 159-165 • Fraser, P.J. (2010) "Pressure Sensing: Depth Sensors and Depth Usage" in Sebert, Phillipe (Ed) "Comparative High Pressure Biology" Science Publishers Inc Enfield, USA. pP143-161

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509 **P 322**

TRIGEMINAL AND SPINAL DORSAL HORN DISCONTINUITY AND AVIAN EVOLUTION

It is generally considered that the sensory trigeminal system is a rostral continuation of the spinal sensory system, evidenced in part by the fact that the concentric spinal dorsal horn laminae of Rexed are replicated in the concentrically laminated trigeminal dorsal horn of the lower medulla. In the majority of avian species (e.g. Gallus gallus), however, the spinal dorsal horn laminae II and III are not concentric, but side by side, with II lying lateral to III (Woodbury, 1998). Curiously, however, this 'schizocerate' condition is not continued into the trigeminal dorsal horn, which maintains a concentrically laminated or 'leiocerate' organization (Puelles et al., 2007). We asked, therefore, where in the chicken spinal cord does the transition from a schizocerate to a leiocerate condition take place, and does the descending trigeminal tract make any contribution to the schizocerate condition of upper cervical segments? These questions were answered by immunohistochemistry and tract tracing of trigeminal and spinal nerves. From an evolutionary perspective, the distribution of both leiocerate and schizocerate morphotypes in most avian lineages suggests that morphology is an inadequate taxonomic marker. Furthermore, it cannot be determined on this basis which morphotype represents the ancestral or plesiomorphic character.

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Neuromodulators

453 **P 323**

Effect of epinastine on acquisition and retrieval of appetitive olfactory memory in the cockroach

Recent findings in insect learning suggest octopamine, a biogenic amine, as a key molecule playing an important role in appetitive learning. We here report that octopamine seems to be involved in appetitive olfactory learning and recall in the cockroach *P. americana*, and the effects of epinastine can be monitored in immobilized animals. In the experiment, cockroaches were restrained to a platform and at the neck, hook electrodes are hooked to the neurons regulating saliva secretion; salivary neurons (SNs). These neurons are known to regulate saliva secretion, and they increase their activities in response to an odor which had been paired with sucrose solution reward in the preceding training, but not to an unrewarded odor. Thus we can monitor the level of olfactory memory by recording the activities of SNs. First, we tested whether an octopamine receptor antagonist, epinastine, inhibits appetitive olfactory memory acquisition. We injected epinastine into the hemolymph before training, and tested the responses of SNs to odors before, during, and after training. Training effect was absent in the epinastine injected group, whereas the control group injected with cockroach saline showed significantly higher level of responses to the rewarded odor after training. Second, we tested whether epinastine inhibits appetitive olfactory memory recall. We injected epinastine into the cockroaches which had been subjected to the same training paradigm. The responses to odors were tested before training, 5 minutes after training, 15 minutes after injection, and 1 hour after injection. Training effect was absent in the test 15 minutes after injection in the epinastine injected group, whereas the control group showed significantly higher level of responses to the rewarded odor in all the tests after training. The results suggest that octopamine is involved in appetitive olfactory learning and recall in the cockroach.

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P 324

Cognitive aspects of insect classical conditioning revealed by studying the roles of aminergic neurons in olfactory and visual memory recall

In insect classical conditioning, octopamine (OA) or dopamine (DA) has been suggested to mediate reinforcing properties of appetitive or aversive unconditioned stimulus (US), respectively. However, the roles of OA-ergic and DA-ergic neurons in memory recall have remained unclear. We studied the roles of OA-ergic and DA-ergic neurons in appetitive and aversive memory recall in olfactory and visual conditioning in crickets. We found that pharmacological blockade of OA and DA receptors impaired aversive memory recall and appetitive memory recall, respectively, thereby suggesting that activation of OA- and DA-ergic neurons and resulting release of OA and DA are needed for appetitive and aversive memory recall, respectively. In order to account for this finding, we propose a new model in which it is assumed that two types of synaptic connections are formed by conditioning and are activated during memory recall, one type being connections from neurons representing CS to neurons inducing conditioned response (CR) and the other being connections from neurons representing CS to OA- or DA-ergic neurons representing appetitive or aversive US, respectively. The former is called stimulus-response (S-R) connection and the latter is called “stimulus-stimulus (S-S) connection” by theorists studying classical conditioning in higher vertebrates. Our model predicts that pharmacological blockade of OA or DA receptors during the first stage of appetitive or aversive second-order conditioning does not impair conditioning, respectively. The results of our study with a cross-modal second-order conditioning procedure were in full accordance with this prediction. We suggest that insect classical conditioning involves the formation of two kinds of memory traces, which match to S-S connection and S-R connection. This is the first study to suggest that classical conditioning in insects involves, as does classical conditioning in higher vertebrates, the formation of S-S connection and its activation for memory recall, which are often called cognitive processes.

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P 325

Time-dependent effects of cyclic AMP and octopamine on the pheromone transduction of the hawkmoth Manduca sexta

Octopamine (OA) improves pheromone-dependent mate finding in moths time-dependently and OA levels in the hemolymph vary in a circadian rhythm. Since OA elevates cAMP levels maximally late at night, during the activity phase of Manduca sexta, we examined whether OA and cAMP sensitize olfactory receptor neurons (ORNs). Long-term tip recordings from pheromone-sensitive antennal sensilla were performed at Zeitgebertime (ZT) 22-1, 1-4, or 8-11 (ZT 0 = lights on), while stimulating the sensilla with bombykal every 5 min. During low endogenous OA levels at ZT 8-11 the perfusion with OA increased both the sensillar potential (SP) amplitude and the action potential (AP) frequency, thus, antagonizing a shift to lower AP frequencies during the resting phase. In contrast, 8bcAMP only increased the SP amplitude without affecting the AP frequency at ZT 1-4 and ZT 8-11. During high endogenous OA levels at ZT 22-1 no OA- or 8bcAMP-dependent effects were observed. The OA-receptor antagonist epinastine (EPI) decreased the AP frequency strongly at ZT 8-11 and slightly at ZT 22-1. In addition, at ZT 8-11 OA antagonized an endogenous shift from phasic to tonic bombykal responses. EPI enhanced the endogenous shift at ZT 8-11 and shifted the responses to a tonic distribution at ZT 22-1. In contrast, 8bcAMP did not affect the AP distribution. Furthermore, OA and 8bcAMP antagonized a decrease of the spontaneous AP activity by increasing the spontaneous AP frequency at ZT 8-11. While OA predominantly affected the burst behavior, 8bcAMP increased the number of bursts and single APs to the same extent. We hypothesize that the circadian release of OA prior to the activity phase disadapts the ORNs. Thus, OA restores strong and phasic responses which guarantee a higher temporal resolution of intermittent pheromone signals. Since 8bcAMP did not mimic all OA-dependent effects we assume that the involved OA receptor(s) in ORNs couple to Gs and Gq. [Supported by DFG grants STE 531/13-1 and 20-1]

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The Behavioral Significance of Serotonin and Nitric Oxide Modulation in Manduca Sexta

Neuromodulation allows for animals to adapt to their environment. All animals experience varying physiological states that reflect factors such as time of day, nutritional needs, and mating status. Neuromodulation allows animals to interact with their environment in a context-dependent manner. This phenomenon has been extensively studied within the antennal lobe (the primary olfactory center) of the hawkmoth, Manduca sexta. Two such neuromodulators, serotonin, a classic neurotransmitter, and nitric oxide, an unconventional neurotransmitter, exert significant changes on neuronal transmission on the single cell and circuit level. However, the behavioral significance of this modulation remains elusive. Using a microinjection surgery, we were able to alter serotonin and nitric oxide levels in the antennal lobe and observe behavioral output in mating and learning assays, respectively. Serotonin antagonism was shown to reduce male mothsâ€™ sensitivity to female sex pheromone. Similarly, inhibiting nitric oxide synthase, the enzyme that catalyzes the production of nitric oxide, sharply reduces odor learning and may also inhibit sensitivity to olfactory stimuli. Furthermore, comparable functions of serotonin and nitric oxide may be representative of an interaction between the two modulators. Soluble guanylyl cyclase, the best characterized target of nitric oxide, is expressed in the morphologically distinct serotonergic immunoreactive neuron. Given similar roles and overlapping anatomical expression, serotonin and nitric oxide may interact synergistically.

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426 **P 327**

NO/OA systems mediate aggressive behavior in the crickets

Male crickets Gryllus bimaculatus show intensive aggressive behaviors when they come across conspecific males. The cricket battle starts out slowly and then escalates into a fierce struggle to establish dominant-subordinate relationship. Therefore, the previous agonistic interactions between male crickets had influence over the following behavior of subordinate males. Once a male loses, it would not fight any more in its second encounter with other males, but shows avoidance behavior from the opponent for more than 3 hours. We have investigated neuronal mechanism underlying cricket agonistic behavior. Biogenic amine octopamine (OA) in the brain decreased in both dominant and subordinate animals after fighting. OA levels in the brain of subordinates were significantly less than those of na□Ee and dominants. Pharmacological and behavioral experiments demonstrated that the behavior of subordinates was mediated by NO/cGMP signaling in the cricket brain. The inhibitions of NO/cGMP signaling pathway using NOS inhibitor L-NAME or soluble guanylyl cyclase inhibitor ODQ increased aggressive intensity in subordinate crickets. When L-NAME, a nitric oxide synthase inhibitor, was injected into a head before the first encounter, subordinates showed significantly earlier recovery of aggressive behavior in their second encounter. In contrast, when mianserin or epinastine, OA antagonists, was injected, the subordinates showed significantly slower recovery of aggressive behavior in their second encounter. Pharmacological treatment of the cricket brain using L-NAME and ODQ significantly increased OA level. An NO donor NOR3, on the other hand, significantly decreased OA level in the brain. These results suggest that NO/cGMP signaling could mediate OA system in the brain, which in turn mediates aggressiveness.

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P 328

Soldier specificities of aminergic systems – Insights into the molecular mechanisms of the division of labor in termites

Sociality has been acquired in many insect lineages independently. Termites, the oldest eusocial insects, also show the elaborate sociality, in which morphologically- and behaviorally-differentiated castes communicate and cooperate with each other. Soldiers, the defensive caste specific to termites, are essential for colonies, because termite colonies are always threaten by various predators. They are differentiated from workers, and through the differentiation, the behavioral tendencies against predator invasions are largely changed; i.e. workers tend to escape rapidly into their nest, whereas soldiers typically aggressively attack enemies. This behavioral transition is remarkable, but the neurological underpinnings are completely unknown. Octopamine (OA) is known as a neuroactive substance to adjust aggression levels in various insects. We hypothesized that the high aggressiveness of termite soldiers involves the OA action. Therefore, we first compared the soma sizes and distributions of OA-immunoreactive neurons in the brain and suboesophageal ganglion (SOG) between soldiers and workers in the damp-wood termite Hodotermopsis sjostedti. The results indicated that several somata of dorsal unpaired median (DUM) neurons in the SOG were significantly larger in soldiers than in workers. Intracellular staining of these neurons revealed that some of them projected into mandibular nerves and tritocerebrum. Considering that mandibular muscles control biting and that the tritocerebrum integrates some information including mechanoreception, these projection areas are thought to relate to defensive behavior. Next, we quantified biogenic amines in the brain and SOG. Although the OA levels in both brain and SOG showed no significant differences between castes, the levels of tyramine (TA), the precursor of OA, was significantly higher in soldiers than in workers. These results suggest that TA or OA in enlarged DUM neurons may be involved in the high aggressiveness in soldiers.

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P 329

Individual aggressiveness in the crab Chasmagnathus: influence in fight outcome and modulation by serotonin

In a previous work we found that size-matched Chasmagnathus crabs establish winner-loser relationships that were stable over successive encounters but no evidence of escalation was revealed through fights. Here, we evaluated the hypothesis that size-matched fights between these crabs would be resolved according to the contestants´ level of aggressiveness. Moreover, we aim at analysing the proximate roots of aggression, addressing the influence of the biogenic amine serotonin (5HT) in crab's agonistic behaviour. To achieve these purposes, the following experiments were carried out. First, we performed successive fight encounters between the same opponents, varying the number of encounters and the interval between them, to assess the stability and progression of the winner-loser relationship. Then, we analysed dominance relationships in groups of three crabs, evaluating the emergence of linearity. Thirdly, we examined the effects of 5HT injections over the fight dynamics and its result. Our findings show that contest outcome is persistent even through four encounters separated by 24 h, but a comparison between encounters does not reveal any saving in fight time or increase in the opponents disparity. Within a group of crabs, a rank-order of dominance is revealed which is reflected in their fight dynamics. Interestingly, these results would not be due to winner or loser effects, suggesting that fight outcome could be mainly explained as resulting from differences in the level of aggressiveness of each opponent. Moreover, this individual aggressiveness can be modulated by the biogenic amine 5HT that increases time allocated to dominant acts.

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247 **P 330**

Species differences in serotonergic neuromodulation of homologous neurons related to locomotor behaviour

We propose that species differences in neuromodulation can cause circuits composed of homologous neurons to produce divergent outputs. To test this, we examined whether serotonergic neuromodulatory actions are conserved across Nudipleura molluscs with similar and disparate locomotor behaviors. In the dendronotid, *Tritonia diomedea*, it was previously shown that serotonin (5-HT) increases the strength of synapses made by Cerebral Neuron 2 (C2). C2 is a member of the central pattern generator (CPG) underlying swimming in *Tritonia*. Evidence suggest that serotonergic neuromodulation of C2"s synapses is necessary for the CPG to generate the swim motor pattern. The pleurobranchomorph, *Pleurobranchaea californica*, can swim like *Tritonia* and its swim CPG contains a homologue of C2 that exhibits a similar firing pattern and synaptic connectivity. There is heterogeneity in the *Pleurobranchaea* population, however; only 20% of individuals swim when tested. We found that 5-HT modulated C2 synapses only in individuals that swam. The aeolid, *Hermissenda crassicornis*, is more closely related to *Tritonia*, but does not swim in the same manner as *Tritonia*. We identified a C2 homologue in this species and found that 5-HT did not affect the amplitude of C2-evoked synaptic potentials. In contrast to differences in serotonergic neuromodulation, we found the C2 synapse with Pedal Cell 5, an efferent neuron involved in crawling, was present in several Nudipleura species. Thus, inter- and intra-species differences in serotonergic neuromodulation correlated with the ability to generate the *Tritonia* type of swimming behavior, but synapses related to a shared crawling behavior were similar across species. The results indicate that divergent behavior could have evolved via the acquisition or loss of neuromodulatory actions. This type of change may allow circuitry controlling conserved behaviors to be unaffected while novel behavior is produced from the same set of neurons.

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P 331

EXTRASYNAPTIC EVENTS IN MOTOR PROGRAM GENERATION

While investigating the cellular mechanisms underlying pharmacological activitaion of motor rhythms by monoamine precursors in a model gastropod Lymnaea stagnalis, we found that, in isolated monoaminergic neurons, the immediate precursor of dopamine or serotonin causes enhancement of extrasynaptic release of the respective neurotransmitter. This release was found to activate extrasynaptic autoreceptors thus affecting signficantly the electrical activity of an isolated neuron (Dyakonova et al., J.Comp.Physiol.A, 2009). Notably, the direction of this effect (de- or hyperpolarization) was in agreement with a specific role of tested monoaminergic cell in the activated motor program. Further, we found that pharmacological or spontaneous activation of motor rhythm within the pedal or buccal ganglia that are responsible for locomotor and feeding behaviors, respectively, can be detected by a multireceptor biosensor (isolated neuron) placed near the ganglion surface. The results suggest that extrasynaptic release accompanies motor rhythm generation and may coordinate the ordered activity of individual neurons with that of the entire pattern generating neuronal ensemble. Grant RFBR 08-04-00120.

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P 332

Molluscan insulin-related peptide evokes long-term potentiation in the *Lymnaea* brain

The pond snail *Lymnaea stagnalis* is capable of learning conditioned taste aversion (CTA) and then consolidating this learning into long-term memory (LTM). Previous studies showed that the molluscan insulin-related peptide II (MIP II) was up-regulated during the CTA-LTM. We thus hypothesized that MIP II plays an important role in the consolidation process for the LTM. To examine this hypothesis, 1) we observed the distribution of MIP II and MIP receptor and determined the amounts of these mRNAs in the central nervous system (CNS) of *Lymnaea*; and 2) we investigated the effects of CNS secretions that are expected to include MIP II on the strength of synaptic transmission that is thought to underlie the CTA-LTM. The MIP II was contained in the cerebral ganglia as previously reported, but the MIP receptor was found to distribute in the whole CNS including the buccal ganglia. Further, the application of a mammalian insulin or secretions from MIP II-containing cells evoked long-term potentiation at the synapses between the cerebral giant cell (i.e. a key neuron for CTA) and its follower B1 cell (i.e. a buccal motoneuron). These results suggest that MIP II triggers the synaptic changes for the consolidation from the CTA learning to its LTM in a snail.

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P 333

Opposing regulation of the oscillatory activity by FMRFamide and serotonin in the slug olfactory center

In the olfactory center of terrestrial animals, changes in the oscillatory frequency of the local field potential (LFP) are thought to be involved in the olfactory-based behavior and olfactory memory. The terrestrial slug Limax velentianus has a highly developed olfactory center, the procerebrum (PC), in which the spontaneous oscillatory activity of the LFP (~0.7 Hz) is recorded. Although changes in the oscillatory frequency are thought to correspond to the preference for specific odors, our knowledge about the mechanism of this frequency regulation is limited. To clarify the mechanism of the bidirectional frequency changes in the PC, we focused on the neuropeptide Phe-Met-Arg-Phe-NH2 (FMRFamide), which is known to have neuromodulatory functions in invertebrate nervous systems. Application of FMRFamide decreased the spontaneous synaptic currents in the PC neurons and the oscillatory frequency via G-protein-mediated cascades. Immunohistochemistry showed that FMRFamide immunoreactive neuronal cell bodies are located in the cell mass layer of the PC, projecting their neurites to the neuropile layers. According to their morphological and projection characteristics, FMRFamide-containing neurons belonged to the subpopulations of both bursting and nonbursting neurons in the PC. Furthermore, the PC was shown to receive FMRFamidergic innervation from other regions of the cerebral ganglion as well. Taking into account previous results showing that serotonin increases the oscillatory frequency, our results indicate that FMRFamide and serotonin regulate the LFP frequency but in exactly the opposite direction in the olfactory center of the terrestrial slug.

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587

P 334

Neuronal nitric oxide synthase in the central nervous system of the pond snail *Lymnaea stagnalis*

Nitric oxide (NO) is a gaseous signaling molecule that functions in numerous biological mechanisms, including neurotransmission or synaptic modification in the central nervous system (CNS). In vertebrates, NO synthase (NOS) proteins are classified into three groups, referred as inducible NOS (iNOS), endotherial NOS (eNOS) and neuronal NOS (nNOS), respectively. The nNOS is different from other NOS proteins in possessing a PDZ domain, and this domain is essential for nNOS function at the synaptic site via anchoring it with other postsynaptic proteins. In the CNS of the pond snail *Lymnaea stagnalis*, previous reports showed the regulatory role of NO in feeding behavior and memory formation, and showed NOS enzyme activity was mainly in the neuropil by NADPH-diaphorase histochemistry. These findings are consistent with the idea that *Lymnaea* NOS proteins are expressed as neuronal NOS, however, none of gene encoding PDZ domain-containing NOS has been isolated in the *Lymnaea* CNS. To analyze the NOS function involved in synaptic regulatory mechanism in *Lymnaea*, we first identified neuronal NOS-like gene (LymNOS3). The deduced amino acid sequence (1645 aa) of LymNOS3, including a PDZ domain, was similar to other species□f nNOS. We next performed *in situ* hybridization in the CNS and quantitative RT-PCR of LymNOS3 mRNA in isolated ganglia or in isolated single cells. The results showed that LymNOS3 gene expression was especially high in the cerebral and buccal ganglia and those ganglia are involved in generating feeding behavior. More, the expression was limited to a particular subset of neurons, including identified neurons such as B2 motor neuron. We also compared LymNOS3 gene expression pattern with the staining pattern for NADPH-diaphorase histochemistry. As a result, the gene expression was consistent with the NOS enzymatic activity. These findings indicate that our newly identified LymNOS3 is functional in the CNS of *Lymnaea stagnalis* and that it has a role in feeding regulatory mechanism.

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 P 335

Organization of the procerebrum in terrestrial snails (Helix, Limax): cell mass layer synaptology and its serotonergic input system

The procerebrum has been considered the center of olfactory information processing/learning in terrestrial snails. Its general neuroanatomy and cellular organization has been known for a while, yet, a number of questions remained open concerning its synaptology. Therefore, this time, we performed a detailed analysis on the cell mass layer of the procerebrum of Helix pomatia and Limax valentianus, aiming at the specifics of the synaptic contacts established in loco. The procerebral cell mass was shown to contain small size neuropils, localized among and surrounded by cell bodies. The axonal elements of the local neuropils formed both specialized and unspecialized axo-somatic and axo-axonic contacts. Simple forms of synaptic configurations also occurred, such as synaptic divergence and presynaptic modulation. The findings are indicative of the role of local circuits in regulating olfactory integrative events, outside the medullary neuropil. Serotonin (5-HT)-immunohistochemistry performed on Helix procerebra revealed a dense perisomatic basket-like innervation of the cell bodies. At ultrastructural level, 5-HT-IR elements were found to contact both cell bodies, and unlabeled axon profiles in the local neuropils. By this we have provided the first ultrastructural evidence for 5-HTergic synaptic inputs in the procerebrum. These investigations were coupled with biochemical assays on the aminergic system, following the application of different odor stimuli. According to HPLC, attractive odor stimulus evoked a limited decrease of 5-HT (22%) and a strong reduction of DA (49%) level in the Helix procerebrum, meanwhile repellent stimulus had an inverse, monoamine increasing effect (5-HT - 64%, DA – 58%). Odor stimulus combined with food initiation (tasting) resulted in a marked increase (84%) of the 5-HT concentration. Upon the use of both attractive and repellent odor stimuli cAMP concentration increased in the procerebrum. In the isolated procerebrum, 5-HT or LSD resulted in a 300-500% increase of adenyl cyclase activity, indicating the positive coupling of 5-HT to the second messenger system. Our findings contribute to a better understanding of the functional-morphological and biochemical basis of olfactory processing in terrestrial snails. Support: OTKA, No. K78224 (K.E.), KAKENHI/JSPS, No. 19370030 (E.I.).

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595 **P 336**

Social interactions determine postural network sensitivity to 5-HT

The excitability of the leg postural circuit and its response to serotonin (5-HT) were studied in vitro in thoracic nervous system preparations of dominant and subordinate male crayfishes. We demonstrate that the level of spontaneous tonic activity of depressor and levator motoneurons (MNs) (which control downward and upward movements of the leg, respectively) and the amplitude of their resistance reflex are larger in dominants than in subordinates. Moreover, we show that serotonergic neuromodulation of the postural circuit also depends on social status. Depressor and levator MN tonic firing rates and resistance reflex amplitudes were significantly modified in the presence of 10 μM 5-HT in dominants but not in subordinates. Using intracellular recording from depressor MNs, we show that their input resistance was not significantly different in dominants and subordinates in control conditions. However, 5-HT produced a marked depolarization in dominants and a significantly weaker depolarization in subordinates. Moreover, in the presence of 5-HT, the amplitude of the resistance reflex and the input resistance of MNs increased in dominants and decreased in subordinates. The peak amplitude and the decay phase of unitary EPSPs triggered by sensory spikes were significantly increased by 5-HT in dominants but not in subordinates. These observations suggest that neural networks are more reactive in dominants than in subordinates, and this divergence is even reinforced by 5-HT modulation.

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P 337

Control of female grasshopper reproductive behaviour by NO-mediated regulation of juvenile hormone release in corpora allata and central brain

Nitric oxide (NO) and juvenile hormone (JH) both influence the reproduction related behavior of female grasshoppers. While JH release from the corpora allata is necessary to establish copulatory readiness including female sound production release of NO in the central complex suppresses female singing. Studies on various insects found NADPHdiaphorase activity in the corpora allata and allatotropin or allatostatins expressing brain neurons in close vicinity of NO generating cells. Both findings suggested the possibility that both NO- and JH- signaling systems may directly interact to mediate a coherent regulation of reproductive states. In female grasshoppers Chorthippus biguttulus different reproductive states ("primary rejection", "copulatory readiness", "secondary rejection after mating") were associated with JH titers in the hemolymph and systemic pharmacological inhibition of NO production induced a reduction of JH content. The possibility of NO signaling in the corpora allata was substantiated by labeling both NO releasing parenchymal cells with anti citrulline antibodies and NO responsive neurons by anti cGMP immunocytochemistry. Accumulation of citrullin and cGMP was inhibited by aminoguanidine, suggesting that it emerged from NO-synthase activity. Backfill studies revealed, that cGMP-accumulating fibers in the corpora allata originated from RFamide-immunopositive brain neurons in the dorsal pars intercerebralis and pars lateralis. This suggests that NO may function as a retrograde transmitter in the CA which gives feedback to RFamide releasing fibers that control the production of JH. Combined immunostaings against Diploptera punctata allatostatin 7 (Dip-Ast-7), Manduca sexta allatotropin (Mas-AT) and components of the NO/cGMP system were performed in the brain in order to identify possible sites where these signaling systems may converge.

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P 338

Mapping male courtship circuitry in Drosophila: GR32a neurons contact OA neurons in the subesophageal ganglion

Identification of the neural circuits that coordinate the expression of innate sex-specific behaviors in response to environmental sensory cues remains a challenge. We previously demonstrated that key octopamine (OA) neurons in the Drosophila subesophageal ganglion (SOG) modulate the behavioral response of male flies to sensory information. Specifically, males without OA neuron function exhibited elevated courtship behavior towards other males in both courtship and aggression paradigms (Certel et al., 2007). The SOG is the primary taste-processing center in the fly and it has been postulated that OA function is necessary to relay contact gustatory pheromone information. Males with impaired Gustatory Receptor 32a (Gr32a) function also exhibit enhanced male-male courtship (Miyamoto and Amrein, 2008). In this study, we examined males with either impaired or enhanced Gr32a neuron function in both aggression and courtship assays. Male-male courtship is elevated in males without Gr32a function, as previously reported; in addition our results indicate a significant reduction in aggressive behavior. To begin to examine how pheromonal sensory information is integrated into social behavior circuits, we used the GRASP dual binary expression system (Feinberg et al, 2008) to detect possible cell-cell connections. In this method, re-combination of two split-GFP parts on the outer membranes of neurons identifies putative synaptic contact sites. We find that the axons of Gr32a neurons contact OA neurons in the subesophageal ganglion, and provide evidence that these contacts are synaptic in nature. Exploring the connections of Gr32a and OA neurons should provide insight into: (i) how two opposing complex innate behaviors are wired in adult nervous systems; and (ii) how sensory information and neuromodulatory neurons coordinate the output of neuronal ensembles to generate distinct behavioral patterns. (Supported by grants from NIGMS to EAK, an NIH COBRE grant P20RR015583 to SJC, and a Pew grant to MF).

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Prozac and its effects on male betta courtship

Some evolutionary biologists propose that elaborate courtship displays in animals may have evolved from aggressive displays between males. Some species show a striking similarity between courtship and aggressive displays, with the only noticeable difference being the sex of the individual at which the display is directed. Fluoxetine, also known by its pharmaceutical brand name Prozac, is one of the most widely known and prescribed antidepressants in the United States. Fluoxetine is an SSRI which blocks the reabsorption of serotonin, and is known to decrease aggressive displays in male Betta splendens, an extremely aggressive fish species. Since many patients taking Prozac report sexual side effects, we hypothesized that fluoxetine might also decrease courtship behavior in bettas. To test this we observed behavioral displays in 32 male betta fish, half of which were treated with 6ig/mL of fluoxetine. We observed how fluoxetine affected courtship behaviors of each male betta fish by measuring the latency of each betta to respond to the female through the glass divider, as well as record and observe the number of courtship behaviors which include: flares, 90° turns, broadside turns, and frontal displays. Fluoxetine had a statistically significant effect in reducing courtship displays in male bettas. These results illustrate the importance of serotonin signaling systems in courtship behavior, and indicate that both courtship and aggressive displays are likely to share the same neural circuitry.

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Sensorimotor integration

667 **P 340**

A mechanical/sound sensitive centrifugal neuron in the central olfactory pathway of the heliothine moth

The antennal lobe (AL) is the primary processing center for odor information in insects. This region contains a large number of sensory axon terminals making synapses with central neurons in characteristic glomerular structures. In addition to the two most numerous populations of AL neurons, the projection neurons and the local interneurons, a third category occur in relatively small numbers — the centrifugal neurons (CN). The CNs extend their dendrites in different areas of the nervous system and send an axonal projection into the AL. CNs are assumingly involved in modulating the afferent information flow in the AL, often via release of biogenic amines. By receiving input from various sensory channels, these feed back neurons are in general presumed to enhance or suppress odor guided behavior according to environmental factors. One extensively studied CN is the so-called VUMmx1, shown to facilitate the formation of memory in the honey bee. Thus, this octopaminergic neuron mediates information about the unconditioned taste stimulus in associative olfactory learning (Hammer, Nature 1993). In order to achieve knowledge about other modulatory networks, e.g. those connected to color and sound, it is necessary to characterize CNs physiologically and morphologically. Here we present a novel CN in the central olfactory pathway of the heliothine moth, identified by the intracellular recording and staining technique. The uni-lateral neuron which has its soma in the dorso-medial region of the PC projects into the AL where it gives off relatively solid branches with blebby terminals in all glomeruli. A second branch innervates the medial PC with many fine arborizations. We have identified the neuron in two heliothine species, Heliothis virescens, and Helicoverpa armigera. Interestingly, the neuron responded to air puffs applied to the antenna and to sound derived from valves when they open and close. The frequency spectrum of the sound includes that of an echolocating bat. The work was supported by The Norwegian Research Council, project number 178860/V40.

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P 341

Interacting sub-systems facilitate visuo-olfactory integration during flight

Interacting sub-systems facilitate visuo-olfactory integration during flight Adult fruit flies excel at locating and tracking odor plumes during flight despite limited visual resolution. Though they have insufficient spatial resolution for object recognition, fruit flies possess rapid visually-mediated reflexes in response to specific patterns of optic flow. The fly optomotor response, analogous to the opto-kinetic response in human vision, robustly compensates for unintended deviations in course. In response to expanding optic flow, flies execute 90˚ turns called body saccades. However not all saccades are triggered by sensory cues. When searching for an odor, saccades punctuate movement lengths that are distributed as a power law, which results in a mathematically optimal strategy for locating sparse resources in the absence of sensory cues. Upon encountering an attractive plume, saccades are suppressed and flies surge upwind and maintain a constant forward heading. How visual and olfactory cues are integrated for the switch between sensory independent search and sensory depending plume tracking is not understood. We have found that olfactory input alone is not sufficient for plume tracking under diurnal conditions. Instead, flies must integrate optic flow information for effective plume tracking. We have further characterized several behavioral algorithms contributing to visuo-olfactory integration. First, the presence of odor increases the gain of the optomotor reflex in the yaw plane, thus directly facilitating a straight flight trajectory. Second, inter-antennal intensity comparisons contribute to finer scale course corrections once a plume has been acquired. However, without optic flow feedback to correct deviations, the fly cannot maintain a constant trajectory in the plume. We sought the neural circuits involved in both olfactory-mediated optomotor gain modification and the generation of a search strategy independent of odor. By correlating the fly's steering behavior to pseudo-random 'white noise' modulation of optic flow, we are able to quickly and accurately derive a quantitative measure of the fly's optomotor filter, which is enhanced by an attractive odor plume, corroborating an earlier finding. The speed and accuracy of the reverse-correlation technique allowed us to screen fly strains for aberrant olfactory-optomotor phenotypes. We took a candidate approach, targeting the function of the mushroom bodies (MBs) of the fly brain with a combined pharmacological and genetic approach, and looked for strains that failed to show olfactory-optomotor modification. The MBs are the second-order olfactory processing center in Drosophila, and are also heavily implicated in olfactory learning and memory. Using hydroxyurea (HU), we ablated the developing MB neuroblasts at the larval stage. Adult flies lacking MBs had wild-type optomotor filters in the absence of odor, which were unaffected by odor. This result was not due purely to a lost sense of smell, as HU-treated animals were able to locate an odor source in a walking trap assay, albeit with a slower time-course. We supported the HU results with targeted genetic manipulations of the MBs, and collective results suggest that the MBs modify visual reflexes downstream of motion processing circuits. Currently, there are no documented connections between the MBs and the visual system. Further studies are currently underway to more finely dissect relevant sub-circuits within the MBs using the expression of transgenic effectors in defined expression patterns. We reasoned that odor dependent changes in optomotor gain might be related to the distribution of saccades for optimal search. We posited that the internal level of hunger in the animal might affect distribution of inter-saccadic intervals (ISIs). Our results show that whereas the ISI distribution did not follow a power-law in tethered flight, the probability of a long ISI increased as a function of starvation. This would result in hungry flies dispersing further than satiated animals, presumably enabling a higher probability of locating food. Surprisingly, starvation did not alter the olfactory-optomotor filter, implying that optomotor reflexes and the generation of a search strategy are controlled by separate sub-systems. The fly must switch between circuits responsible for generating one set of adaptive behaviors to another depending on sensory and physiological context. In the absence of odor, flies generate saccades spontaneously with a probability that is a function of hunger. Olfactory cues seem to exact an entire suite of changes to enable plume tracking: the contribution of optic flow information to the animal's behavior increases, the gain of the yaw optomotor response increases, and saccades are suppressed. Our ablation studies demonstrate that olfactory information modifies circuits downstream of the MBs, rather than the lateral horn, the other pathway that might carry an olfactory signal. These results indicate that the information carried in the MBs contribute to switching between context-dependent circuits, allowing the fly to produce adaptive behavior in multiple sensory environments.

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P 342

Analysis of flight trajectory of honeybee (Apis mellifera) for the olfactory stimulus in a wind tunnel

Olfactory related behavioral mechanisms of honeybees (Apis mellifera) have been investigated extensively, such as learning mechanisms by the analysis of proboscis extension reflex, transmission of information about forging site. Several studies for olfactory induced orientation flight are conducting in the wind tunnel and the field. In these studies, honeybees utilize optical flow as main information and olfactory signal additionally for navigation. It was also shown that flight trajectory shows Lévy property. However, the detail of flight behavior responded to odor still hard to investigate, so it is not clear how olfactory information is utilized in the dynamic control of flight. Therefore to target the mechanisms of olfactory navigation in honeybees, we analyzed properties of flight trajectory when oriented to an odor source in a wind tunnel. Using a wind tunnel, we recorded flying bees with a video camera to analyze the flight area, velocity, angular velocity and direction. Bees were trained with an artificial feeder with an odor in the center of wind tunnel. After the feeder was removed, the flight behavior was compared under the condition with or without the olfactory stimulus, given from a glass pipe, located upwind from the feeder place. The results showed that the ratio of heading for upwind was increased just after the odor stimulation. The velocity and angular velocity were changed when honeybees closed to odor source, but they were not changed without odor stimulus. In addition, the results showed that these values were significantly different between the inside and the outside of the odor area. Flight trajectories tend to be bent or curve in the just outside of the odor range. We conclude that flying bees responded to odor flow and oriented the odor source to correct that course without deviating from the odor.

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614 **P 343**

Study on sensory feedback during odor searching behavior of silkworm moth using a brain-machine hybrid system

Insects perform adaptive behavior according to changing environmental conditions using their small brains. Here we define adaptability as an ability to execute a task in different environmental conditions. This adaptability is generated through the relationship among brain, body and environment, so it is necessary to examine how a brain process changing environmental information during behavior. To understand neural processing during behavior, we constructed a brain-machine hybrid system using motor signals related to the steering behavior of the male silkworm moth for controlling a mobile robot. Using the hybrid system we can intervene with the relationship of brain and environment by changing parameters of a robot movement. We developed this hybrid system according to the following steps. (1) For electrophysiological multi-unit recordings on a robot, we developed small amplifiers. (2) We selected units that activated during neck swinging induced by optic flow as steering information. (3) To control a robot by neural activities, we implemented a spike-behavior conversion rule such that frequency of the left and right neck motor neurons�f spikes was linearly�@converted into rotation of the wheels. We reproduced the moth�fs programmed behavioral pattern and orientation behavior toward a pheromone source on the hybrid system. We compared the orientation behavior of moths with that of the hybrid system under different pheromone conditions. Moreover, using the hybrid system, we examined adaptability of the silkworm moth. At first, we examined whether moths responded to change of movement programmed to the hybrid system. We observed compensation motor signals in response to the externally given movement change, and this compensation was caused by the sensory feedback through compound eyes. Then we examined how moths responded to change of spike-behavior conversion rules (ex. left or right biased motor gain, increase or decrease of motor gain) during odor searching behavior.

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P 344

Relating neuronal to behavioural performance: State-dependence and other sources of variability of visual-motion induced head movements in the blowfly

Behavioural responses of an animal to repeated presentation of the same stimulus may vary considerably. This variability can arise from stochastic processes inherent to the nervous system. Also, the internal state of an animal may influence a particular behavioural response. Here we investigate to what extent behavioural variability is caused by sensory neurons that are involved in controlling a particular behavioural response and address the question where in the sensory-motor pathway state-dependent changes found at the behavioural level are mediated. We have chosen visually induced head pitch movements of blowflies and their neuronal control by large motion-sensitive neurons, so-called LPTCs in the fly's third visual neuropile, as our experimental paradigm. Only two identified LPTCs in each half of the brain are thought to provide the sensory information for downward head pitch movements that are induced by visual motion. They directly innervate motor neurons [Milde et al., J.Comp.Physiol.A 160:225-238 (1987); Strausfeld et al. J.Comp.Physiol.A 160:205-2224 (1987)] rendering the neuronal pathway from sensory output to motor control especially straight-forward. We combine electrophysiological and behavioural experiments performed under similar stimulus conditions with model simulations to test to what extent the behavioural variability can be predicted on the basis of the sensory information provided by the LPTCs. To account for state-dependent changes neuronal as well as behavioural experiments were performed while the animal spontaneously assumed a high or low motor activity state. During behavioural experiments head movements were monitored by high-speed digital cinematography while the animals were stimulated by visual motion stimuli. Head pitch responses to downward motion are highly variable. Part of the variability can be attributed to two different motor activity states of the animal. Also LPTC responses depend on the fly's motor activity. By means of a simple modelling approach we predict head movements on the basis of the recorded responses of motion sensitive output neurones of the visual system and compare the variability of the predicted with that of the actually observed head movements. The large gain changes of visually induced head movements that go along with changes in the state of motor activity cannot be explained by state-dependent changes in LPTCs. Instead, they are imposed downstream the motion sensitive output neurons of the visual system. We propose that a central signal associated with motor activity changes the gain of visually induced head responses and the response properties of LPTCs [Rosner et al., J Exp Biol 212:1170-1184 (2009); Rosner et al, J Exp Biol 213: 331-338 (2010)]. If a central signal is necessary to change the gain downstream of the LPTCs what then is the function of the state-dependent response changes in LPTCs? One function could be to enable flies to respond fast when moving actively through their environment while saving energy, when it is less important to respond fast. Even within a given motor activity state, the variability of LPTCs does not suffice to explain the large behavioural variability. Hence, not only the gain changes at downstream processing stages but also substantial post-sensory noise is added there.

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P 345

Making an Escape: Coordinating Behavioral modules for flight initiation in Drosophila

How does an animal select and coordinate a series of actions? We have identified a sequence of behaviors that the fruit fly, Drosophila melanogaster, performs in response to a rapidly approaching object or looming visual pattern. These behaviors enable a fly to control the direction of an escape jump that launches it into flight, away from a perceived threat. To investigate whether the suite of behaviors directing escape is a stereotyped sequence or is more flexible, I used high-speed videography to capture the responses of individual fruit flies to computer-generated looming disk stimuli presented on a high refresh rate CRT monitor. I adjusted parameters of the looming virtual disk, such as approach speed, approach duration, or contrast, to make the stimulus more or less salient. I found that flies still perform a subset of the previously observed escape behaviors, regardless of whether or not they take off in response to the stimulus. This indicates that the escape behavioral sequence is not a fixed action pattern. Furthermore, I found that the escape sequence can be divided into separable behavioral modules whose timing and probability of occurrence are differentially affected by changing stimulus parameters. The fly also uses some of these modules when initiating flight "voluntarily" without direct external stimulus. Thus, by breaking up the takeoff sequence into individual action modules, the fly is better able to tailor its takeoff performance to its current circumstance.

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P 346

The role of the central complex in Drosophila locomotor behaviour

The central complex (CX) is an intricate, symmetrical collection of neuropils located on the midline of the protocerebrum that has been observed in several insect species and implicated in several behaviours. We have identified a series of enhancer trap lines expressed in the CX and used them to conduct a neuroanatomical and locomotion study of this region. The CX has intricately organised neuronal structure containing at least 60 different types of neurons. Using enhancer trap lines we have observed several isomorphic sets of both small and large field neurons and have built these and previously observed Drosophila CX neurons into a classification tree. Having identified many CX neuron sets using >20 enhancer trap lines, we then selected those lines that limited expression to various neuron sets in the CX (minimal expression outside the CX) and crossed each line to a UAS-shibirets line. The various Gal4; UAS-shibirets progeny and controls were then subjected to three different locomotor paradigms: 1) Activity monitor 2) Buridan's paradigm 3) Optomotor maze In the activity monitor experiments were conducted in the dark, each fly was alone in a narrow tube for 4 hours. The number of times each fly crossed a centrally placed laser beam was counted automatically. In Buridan's paradigm, a single fly with clipped wings was placed on a circular platform surrounded by a water moat in a brightly lit enclosed arena. Two black stripes on opposite sides stretched the height of the arena and acted as inaccessible landmarks for the fly. Through the use of a webcam the fly was tracked from above for 15 minutes using custom built software. Finally, the optomotor maze consisted of a gravity maze with a series of left or right choice points placed flat over an LCD monitor displaying a grating moving perpendicular to the flies' orientation. Final maze exit points indicated the visual response and optomotor phenotype of each group of flies. We will present the results of this study here.

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P 347

Multi-channel Analysis of Sensory Integration within the Central Complex of the Cockroach

The central complex is a group of prominent, highly structured neuropils that reside on the midline of insect brains. Circuits within the CC have been associated with sensory input and motor control. Our previous extracellular multi-channel recording revealed neural units in the cockroach brain that respond to tactile stimulation of either antenna or visual cues. The extent of these multi-sensory responses led us to wonder how activity from multiple sources was integrated. We compared responses to moving both antennae and to those from individual antennae. The dual antenna responses reflect biases in the component single trials, but are not simple summations. For example, units that respond more strongly to medial antennal movement also are biased toward inward movement of both antennae over outward movement. However, the response to inward movement did not exceed that of single antenna medial responses. We hypothesized that antennal movement generates both excitation and inhibition, so that dual trials would increase both effects with no change in amplitude. To test this, we delayed the stimulus of one antenna by varying times ranging from 120 to 360ms. The delayed response was often significantly reduced relative to single antenna trials. These effects were particularly apparent when we examined the averaged responses to all units that were recorded simultaneously. As the delay increased, a change in response strength often occurred between 140 and 160ms, suggesting a point where excitation and inhibition come out of register. We also examined interactions between visual and antennal stimulation. Visual responses can either be phasic or tonic. Phasic responses are similar to antennal responses. However, tonic visual activity can mask prominent antennal responses in single units. We are also examining visual responses to moving stripes projected onto the visual field. Supported by NSF grant IOS-0845417 to RER.

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Active sampling in locust olfaction

How does the processing of sensory information change when an animal actively investigates its environment? Most of our knowledge about how the insect nervous system encodes olfactory information comes from experiments on restrained animals. However, during normal function, locusts and other insects move their antennae (the main olfactory organs). To determine whether locusts use these antennal movements to actively sample odors, we characterize how antennal movements change in the presence of an odor. In addition, we are currently engaged in analysis of simultaneously recorded neural activity to determine how antennal movements affect the odor responses of olfactory projection neurons. We record the direction of a tethered locust's walking while simultaneously tracking its antennal movements in 3D. We find that, when presented with a spatially localized odor, locusts change the frequency and location of their antennal sweeps, focusing them around the odor location. This behavioral strategy leads to an increase in the frequency at which the locusts sample the odor and biases the odor samples to occur closer to the odor source. To determine the implications of this behavioral strategy for the neural encoding of odors, we record neural activity from the projection neurons while the locust is free to move its antennae to sample odors.

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P 349

Object preference by walking flies, Drosophila melangaster, is mediated by vision and graviperception.

Organisms are not evenly distributed across the surface of the earth. They are found in clusters based on the distribution of resources such as food, mates and shelter. We are studying how walking fruit flies, Drosophila melanogaster, use sensory information to structure their exploratory behavior. We developed a controlled sensory environment in which we could study the fliesâ€™ locomotor behavior as they explored an arena containing four objects of equal lateral surface area but differing height and slope. We found that flies approached these objects with equal frequency and once they reached an object, the flies walked to the top of the objects and performed stops. The flies performed stops of longer duration on the tallest, steepest object in the arena than on the other objects. This change in locomotor behavior underlies the fliesâ€™ innate preference for spending more time on the tallest, steepest object in the arena. Additionally, the assessment of the conesâ€™ geometry is by an absolute measurement during the exploration of the cones rather than comparison to other cones or by memorization of a given coneâ€™s features during approach. We found that the flies were able to use either vision or graviperception (via the antennae) to assess the geometry of the objects. Only when both of these sensory modalities were impaired did the flies not respond to the objects with longer duration stops. In order to further investigate what aspect of object geometry the antennae sense, we created additional landscapes with objects of either equal height or slope. These experiments indicated the fliesâ€™ antennae are able to sense the slope of the objects but not the absolute height. It is likely that the Johnstonâ€™s organs, known gravitational receptors, mediate this perception of slope. However, the experiments to determine the aspect of cone geometry the visual system assessed were inconclusive.

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686 **P 350**

Flight mechanics of visually elicited altitude responses in freely flying Drosophila

Insects, such as Drosophila, are thought to achieve robust flight stability through the implementation of rapid sensory-motor reflexes. Specialized interneurons within their visual system detect unintended rotations and translations during flight and convey that information to motor circuits that generate compensatory changes in wing motion. Until recently, most investigations of sensory motor reflexes in Drosophila have utilized tethered animals, but such conditions are known to generate artifacts. Measuring the subtle changes in wing motion that are elicited by visual input in free flight requires both very high spatial and temporal resolution, and the ability to track a flying animal within a suitably large region of space. To this end, we have developed an arena in which we can present arbitrary visual stimuli to freely flying fruit flies, and record with high definition their behavioral responses using 3D high speed videography. As the insects traverse a small volume in the arena, a laser-based detector circuit triggers a moving visual pattern displayed on a cylindrical panorama of LEDs. By analyzing the open-loop responses of the animals to the visual motion, we are able to characterize with high accuracy the changes in wing and body motion that are elicited during visually-mediated compensatory flight reflexes. The high-throughput in our analysis is facilitated by implementation of automated tracking, which yields Kalman filtered estimates of the 3D kinematics by combining a parameterized generative model of the fly with the reconstructed visual hull from the recorded images. In a preliminary study, we investigated the insect's altitude control mechanism by eliciting responses to vertically displacing gratings displayed on the walls of the flight chamber. When presented with this stimulus, flies adjust their wing motion to generate a syndirectional flight response, which can be interpreted as an attempt to minimize the slip of the pattern on their retina.

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P 351

Blinding speed: Effects of relative motion on insect vision

Anyone who has tried to take a picture of a moving object with a camera set at too slow a shutter speed knows that the resulting image is blurred and generally lacks contrast. The same phenomenon occurs with animal visual systems. If the relative velocity of the observer and the viewed object, say a potential mate or prey item, is faster than the photoreceptors or higher order visual neurons can handle, the resulting perceptual image loses contrast and suffers from "motion blur". While faster moving insects generally have faster visual systems (photoreceptors: Laughlin 1996, Vis. Res. 36:1529-1541; higher order cells: O'Carroll et al. 1996, Nature 382:63-66), whether or not the spatio-temporal response properties of an animal"s eyes are quantitatively adapted to it"s locomotory speed is an open evolutionary question. Some systems are reasonably matched, but others clearly are not. Predatory tiger beetles (Carabidae: Cicindelinae) are cursorial visual hunters typically 1 – 2 cm in body length that run extremely fast after their prey. Some species achieve linear velocities up to 2 m/s and angular velocities of 1500 o/s. These beetles run so fast after their prey that they go blind during pursuit of prey and must frequently stop to relocalize the prey. Computational modeling based on retinal optical measurements and electrophysiological recording of green-sensitive photoreceptors demonstrates that the contrast of the beetle's world is extremely degraded at velocities well below the beetle's maximum running velocity. Nevertheless, the beetles are very successful predators if prey does not fly from the plane of the substrate during the beetle's pursuit. High speed film analysis of running beetles reveals large step lengths and high step frequencies. Whether such kinematics of the locomotory system represent an energetic trade-off with sensory abilities will be discussed.

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P 352

Optomotor and Goal-oriented Turning Behavior in Tethered and Free-walking Cockroaches

As a mobile animal traverses its world, it may encounter barriers that must be identified and negotiated, and doing so is an important function of an animal"s brain. We have refined two behavioral paradigms for studying the neural control of directed locomotion in free-walking and tethered cockroaches, Blaberus discoidalis. First, we have developed a T-maze in which a cockroach approaches an LCD screen displaying a vertical striped pattern. The stripes are triggered as the cockroach reaches the choice point, biasing its turns via the classical optomotor response. Second, we constructed a brightly lit arena containing a dark shelter area. Cockroaches are strongly motivated to seek the shelter in these circumstances. Both of these behaviors are visually mediated, which makes them amenable to more detailed analysis using existing protocols on tethered animals. We placed tethered cockroaches over a Styrofoam ball suspended in a stream of air. The ball"s 3D rotation was monitored in real time using a single camera and optic-flow analysis software. A cockroach"s walking turns were used to control the position of a visual stimulus on an array of 3 LCD monitors. Dark areas on a white background elicited goal-directed steering reminiscent of the shelter-seeking response in the arena. The strength of the responses depended on the size but not the aspect ratio of the attractive, dark stimulus area. Horizontal motion of vertical stripes also resulted in optomotor turning responses, as in the T-maze. Turning behaviors were related to neural activity in the lateral accessory lobes of the brain, premotor areas downstream of the central complex, into which we implanted wire-bundle tetrodes in our tethered animals. This work was supported by AFOSR grant FA9550-10-1-0054 and NSF grant IOS-0845417to RER.

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P 353

Tracking body and leg movements during phonotaxis in Crickets

Cricket phonotaxis is one of the most well studied behaviours in acoustic communication. However, there is still limited information about the precise body and leg movements during the approach to the sound source. Here, we present a new method that allows us to gather this data in relation to sound status and direction. Prior to experiments, we remove the two top wings from adult female crickets and attach a light string to their back, which is used as a tether to keep them in a restricted area. Then we apply small dots of paint on their leg joints and at the centre of the thoracic segments. The insect is placed inside an arena with a transparent base, marked with a grid of points. The walls and ceiling are covered with sound absorbent material to prevent sound reflections. We use two speakers, positioned on the left and right side of the box, to attract the insects, by alternately playing the calling song. Two high speed cameras located under the base capture the movements of the insect, and simultaneously record an LED signal corresponding to the sound pulses. During the experiments we follow the insect by moving the cameras in a XY-plane. We have developed a custom-made software to calibrate the cameras, track the joints and grid positions, determine sound status and direction and stance-swing transitions. The above method results in a full 3D reconstruction of the insect, and allows the investigation of the role of individual joints, legs and thoracic segments during phonotaxis. Consequently, this research will help us understand, at a behavioural level, the transformation of the input sound signal to the motor output of each leg segment. We will use these results to expand previous neural models of phonotaxis and test them on a hexapod in dynamic simulations and robot implementations.

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P 354

Mechanosensory-Visual integration during the honey bee flight

Flying insects require input from multiple sensory modalities while performing aerial maneuvers. These modalities relay sensory information to the nervous system at various latencies. Mechanosensors provide information on a wingstroke-to-wingstroke basis whereas visual feedback is slower due to multiple levels of processing. For fast maneuvers, integration of both these cues is especially important. Our goal is to study how mechanosensory and visual feedback combine during aerial maneuvers. We present preliminary data on how antennal mechanosensors influence flight in honeybees. Bees (Apis mellifera) were trained to fly to a feeder placed at the upwind end of a custom wind tunnel and were filmed using two high speed video cameras. We varied the windspeed within the wind tunnel and recorded the antennal response to changes in wind speed by measuring the interantennal angles. Although interantennal angles were not correlated with the bee's groundspeed (motion of body relative to ground), they decreased as a function of increasing airspeeds (motion of body relative to ambient air flow). Because the antennae respond to changes in ambient air flow but are unlikely to be influenced by motion relative to the ground (optic flow), the data suggest that bees are sensing ambient airflow. Which sensory modalities are involved in airflow sensing and antennal positioning? To address this,we are exploring the roles of antennal mechanosensors (e.g. Johnston's Organs and Bohm's bristles) which have been previously implicated in airflow sensing. When Bohm's bristles located on the base of the antenna are ablated, the bees misposition their antenna and also show an inability to sustain flight. Flight ability is affected when input to the Johnston's organs is removed by amputation of antennal flagella, however it is recovered partially when the flagella are reattached. Thus, similarly to moths (Sane et al 2007), mechanical integrity of the antennae is essential for flight control.

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Estimating Head Orientation of Honeybees from Body Yaw Angle

Many navigation capabilities of honeybees, e.g. the return to a food source or obstacle avoidance, rely on vision. For better understanding of the underlying mechanisms it is advantageous to reconstruct the visual input bees experience during flight. This, however, makes it necessary to know head orientation which can be determined only from close-up video footage, considerably limiting the area over which trajectories can be recorded. In order to improve this situation we attempted to infer head orientation of bees from body yaw angle. During flight, honeybees move their heads in a saccadic way: Fast yaw turns (saccades) are followed by periods where head orientation is kept almost perfectly constant (Boedekker et al., 2010). Using close-up high-speed video recordings from which head and body orientation could be simultaneously measured, we derived rules for predicting head saccade events from peaks in body yaw velocity. For estimating head orientation from body yaw angles, an appropriately scaled "saccade template" is inserted at these points in time. Between the predicted saccades, head orientation is estimated by averaging the body yaw angle. For the "saccade template" we employed a sigmoid function whose parameters were determined by fitting the time course of head saccades of various amplitudes. References: N. Boeddeker, L. Dittmar, W. Stürzl, M. Egelhaaf (2010). The fine structure of honeybee head and body yaw movements in a homing task. Proc. R. Soc. B. 277, 1899-1906.

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P 356

Honeybee flight: A novel "streamlining" response

Using virtual reality, an experimental paradigm has been developed to investigate visually guided insect flight and navigation using tethered honeybees. Simulation of forward image motion, characteristic of forward flight, elicited a novel 'streamlining' response in which the abdomen is held in a raised position. This visually evoked response was observed in the absence of any airflow, and is thus driven only by optic flow. The 'streamlining' response (raised abdomen) may serve to reduce the aerodynamic drag that would otherwise be produced by the abdomen during real flight. The strength of the 'streamlining' response was highly dependent on stimulation of the entire visual field of the honeybee. This highlights the importance for a totally panoramic stimulation for the study of visually guided flight in insects.

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Flight motor responses to looming visual stimuli in tethered flying bumblebees, *Bombus ignites*

To avoid impending collisions successfully, insects must detect particular features of approaching objects and recruit the motor systems within a limited time to collision. Flying insects rely on vision for flight control, therefore characteristic visual-motor systems for collision avoidance would have evolved. In the central nervous system of locusts, looming sensitive neurons (LGMD and DCMD) are thought to be involved in triggering collision avoidance behaviors, however, there are few studies reporting similar systems in other insect species. In this study we focused on the visual-motor system in bumblebees, which can perform sophisticated flight maneuvers with high wingbeat frequency powered by myogenic flight muscles. We applied looming stimuli (projected images of an expanding black circle on white background) to tethered flying bumblebees, *Bombus ignitus*, employing a customized high-speed projector using a digital mirror device (500-5000 fps). As behavioral responses to the stimuli, we measured electromyograms (EMGs) of the indirect wing depressor muscles (dorsal longitudinal muscles: DLMs), wingbeat frequency, leg and body movements. We observed that EMG frequency of the DLMs increased with increasing size of the looming object and decreased rapidly before end of the stimuli. Timings of onsets of the increases in EMG frequency and its peaks were correlated linearly with projection speed (i.e. approaching speed) of the object, therefore timings of the changes in the DLM motoneuron activities were triggered by particular object sizes. We also observed that wingbeat frequency also increased with increasing EMG frequency of the DLM. The response properties of the DLM EMG frequency were similar to those of relevant locust neurons, it is therefore likely that the DLM is driven by corresponding looming sensitive neurons enabling bumblebees to rapidly change wingbeat frequency.

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P 358

Predator avoidance by locusts in a swarm

Locusts have evolved a high level of sensitivity to looming objects, probably as an adaptation to predation and a swarming life style. This sensitivity is mediated by two large identified neurons: the lobula giant movement detector (LGMD), and its postsynaptic target, the descending contralateral movement detector (DCMD). These neurons respond most strongly to rapidly approaching objects. The DCMD excites flight motoneurones and can trigger predator avoidance during flight: in response to a looming stimulus a tethered, flying locust will perform a 'gliding dive', in which it ceases to beat its wings for one or more wingbeat cycles; the wings are held elevated in stereotyped gliding posture. These gliding dives are evasive maneuvers and only occur in response to approaching objects when a DCMD produces consecutive spikes at a frequency of at least 150 Hz during the elevation phase of a wingbeat cycle (Santer R.D et al J Neurophysiol 95 3391-3400, 2006). When the locust is in an aroused state visual processing by the LGMD is altered in particular the prolonged periods of high frequency that trigger evasive glides are enhanced. Applying a brief mechanical stimulation to the hind leg or flight itself causes the DCMD response to recover from a previously habituated state, so that it is significantly more likely to generate the maintained spike frequencies capable of evoking gliding dives even with extremely short intervals (1.8 s) between approaches. In tethered flying locusts 41% responded with a glide to 6 images of approaching objects, separated by 1.8s. Octopamine may be responsible for the effect because injecting the neuronal octopamine receptor antagonist, epinastine, into the haemolymph reduced the number of glides from 41% to 12%. Consistent with this role ultrastructural studies show putative octopaminergic terminals, identified by their dense core granules, in close proximity to the input dendrites of the LGMD in the lobula.

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695 **P 359**

Visual control of altitude in flying Drosophila

Unlike creatures that walk, flying animals need to control both their horizontal motion as well as their height above the ground. Extensive work on insects, the first animals to evolve active flight, has revealed several visual reflexes that are used to govern horizontal course. For example, flying insects orient toward prominent vertical features in their environment and generate compensatory reactions to both rotations and translations of the visual world. Insects also avoid impending collisions by veering away from visual expansion. In contrast to this extensive understanding of the visual reflexes that regulate horizontal course, the sensory-motor mechanisms that animals use to control altitude are poorly understood. Using a 3D virtual reality environment in which we could automatically track flies and present them with visual patterns, we found that Drosophila utilize three reflexes – edge tracking, wide-field stabilization, and expansion avoidance – to control altitude. By implementing a dynamic visual clamp, we found that flies do not regulate altitude by maintaining a fixed value of optic flow beneath them as suggested by a recent model. The results identify a means by which insects determine their absolute height above the ground and uncover a remarkable correspondence between the sensory-motor algorithms used to regulate motion in the horizontal and vertical domains.

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P 360

Flight muscle timing, wing kinematics and 3-dimensional body orientation of loosely tethered locusts responding to looming objects

In locust flight, collision avoidance behaviour involves integration of visual sensory cues with coordinated, bilateral, wing muscle activity. Previous studies examining wing and body kinematics used open loop conditions that prevented generation of realistic steering torques in all three rotational degrees of freedom (pitch, roll and yaw). Recently, motor neuron activity in rigidly tethered locusts has been related to the initiation of a glide as a last ditch avoidance manoeuvre (Santer et al. 2006). Collision avoidance responses of loosely tethered locusts, however, suggest that this behaviour is more complicated and subtle that previously thought (Mohr and Gray 2003). We placed locusts in a wind tunnel using a loose tether design that allowed for motion in all three rotational degrees of freedom during presentation of a computer-generated looming disc. Two high speed video cameras (250 fps) recorded flight responses and allowed us to extract wing kinematics, abdomen position and 3-dimensional body orientation. Concurrent EMG recordings monitored bilateral activity from the first basalar depressor muscles (M97) of the forewings, which have been implicated in flight steering. Behavioural responses to a looming disc included cessation of flight (with wings folded over the body), glides and active steering during sustained flight. Active steering involved shifts in bilateral m97 timing, wing asymmetries and whole body rotations in the pitch, roll and yaw planes. On the inside of a turn, m97 activity occurred earlier relative to the top of the wing stroke and bilateral timing corresponded to forewing asymmetry as well as changes in whole body rotation. These data provide information on mechanisms underlying 3-dimensional flight manoeuvres and will be used to drive a closed loop flight simulator to study responses of motion-sensitive visual neurons during production of realistic behaviours. Mohr and Gray (2003) NeurosciAbstr Program number 403.20, Santer et al. (2006) J Neurophysiol 95:3391-3400.

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P 361

Identifying flight stabilizing mechanosensors in the Eastern bumblebee (Bombus impatiens)

Insect flight requires multimodal sensory information processing for movement control. In flies, visual information combines with gyroscopic mechanosensory information to maintain a flight trajectory. This gyroscopic information can be provided by halteres, which encode torque produced by body rotations. In many insects, however, sources of information for flight control are unknown. This study investigated whether Eastern bumblebees (Bombus impatiens) might have structures which behave similarly to halteres. To identify structures that may be used to stabilize flight, we ablated one of three putative flight-stabilizing mechanosensory structures, the antennae, hindwings, and tarsi, and observed performance during perturbed flight. While bees were flying, we removed visual light and recorded flight behavior under infrared light, using high speed videography. By digitally tracking points on the body in the videos, we compared flight stability with lights on and duration of stable flight after lights off for intact animals and each of the three ablations. After the lights are shut off, bees were able to remain airborne for a short period of time (mean 795.5 ms) before falling from flight. The flight performances of separate groups of flying bumblebees were tested after removal of one of the three putative flight-stabilizing structures. Latencies to fall from flight were found to be significantly shorter in ablated than control trials (1417 vs. 345 ms). This finding suggests that one or a combination of the three structures provide mechanosensory information required for stable flight. We found no significant effect on body stability or velocity during flight under visible light along the three axes of rotation (yaw, pitch, roll), which suggests that ablation does not affect flight with visual input. Our results suggest that insects have a multitude of structures, endowed with mechanosensors, which could provide gyroscopic information required for flight control.

Zamore, Sharri

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Visual flight control in a nocturnal insect

To avoid collisions when navigating through cluttered environments, flying insects must control their speed so that their sensory systems have time to detect obstacles and evade them. Day active insects are known to rely primarily on visual cues to regulate their flight speed in cluttered environments. They do this by holding constant the speed of the visual scene as it moves across the eye. However, many flying insects are active at night, when obtaining reliable visual information for flight control presents much more of a challenge. Do nocturnal flying insects also rely heavily on visual cues to regulate their flight speed in dim light? To address this question, we measured the speed of the nocturnal neotropical sweat bee, Megalopta, flying along an experimental tunnel when the visual texture on the walls was a check pattern, providing strong horizontal (front-to-back) visual motion cues, a stripe pattern, which provides no horizontal motion cues, or a combination of these two (i.e. each wall displayed a different pattern). When horizontal motion cues in the tunnel are minimised, Megalopta fly approximately three times faster than when these cues are strong. These findings demonstrate that Megalopta, like their day active counterparts, do indeed rely heavily on vision to control flight speed, despite the difficulty of doing so under extremely dim light conditions. Surprisingly, the flight speed of bees flying in the tunnel when each wall displays a different pattern lies halfway between the speed observed in the check condition and that observed in the stripe condition. This is what would be expected if Megalopta were regulating their flight speed by averaging the apparent rate of horizontal visual motion over the entire visual field, a behaviour that has not previously been observed in any other flying insect and has important implications for our understanding of how vision is used to control flight in insects.

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Guidance laws underlying prey capture in the dragonfly

Dragonflies are nature's consummate aerial predators; flying at extremely high speeds, they catch small moving insect prey; escapes are rare. We have constructed the first indoor dragonfly flight arena in order to unravel the computations and circuit dynamics underlying this remarkable behavior. We will present data from an array of high speed cameras that allows us to reconstruct the trajectory of the dragonfly and its prey (drosophila). From these data, we can test quantitatively the hypothesis (Olberg et al., 2000) that dragonflies use a strategy of proportional navigation to intercept their prey. Starting with the three-dimensional coordinates of the dragonfly and its prey, we calculate the azimuthal and elevational line-of-sight position of the prey as a function of time. The numerical derivatives of these line-of-sight vectors are then estimated, and their drift rates are compared to the dragonfly's acceleration vector normal to its bearing. The extent to which these numbers are consistent with different models of proportional navigation and other guidance laws will be discussed.

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Frequency Dependence of Prey Localization Responses in the Leech Hirudo Medicinalis During Different Life Stages

Many aquatic predators such as frogs, leeches, and predatory fish are able to localize their prey by orienting toward disturbances in the water which their prey makes (Elepfandt, 1984; Horridge and Boulton, 1967; Müller and Schwartz, 1982; Young et al., 1981). Here, we examine the ability of the leech to perform this task under a variety of conditions. Our data suggests that behavioral responses to waves are frequency dependent. For instance, adult leeches are better able to localize the source of a 4 Hz water disturbance than a 1 Hz disturbance. However, prey preferences in leeches change over the leech's lifetime (Keim, 1993; Wilkin and Scofield, 1990). While juveniles feed on frogs, adults prefer to feed on mammals and even have higher reproductive viability when they do so (Sawyer, 1983). These animals may not only be able to localize prey by sensing water waves, but also, could use this information to determine the type of prey. Does this change in prey preference correspond to a change in the leech's response to different frequencies of water waves? We have found that juvenile leeches exhibit a greater behavioral response to lower frequency stimuli than their adult counterparts. Interestingly, leeches have two sensory modalities capable of sensing water waves: mechanoreceptors on their skin, or their array of more than 300 simple eyes. However, these modalities differ in their sensitivity to stimuli of specific frequencies. Neurophysiological recordings have shown that these sensors exhibit a differential response to wave stimuli. While the visual sensors respond best to low stimulus frequencies, the mechanosensors are much more broadly tuned (Friesen, 1981; Kretz et al., 1976; Peterson, 1984). Preliminary behavioral data in adults shows a similar shift in response toward lower frequencies when only visual information is available. In the future, we hope to examine how this information is processed at different levels within the nervous system.

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Sensory processing by a nonspiking neuron in the leech

Processing of sensory signals into appropriate behavioral responses is accomplished at several levels throughout sensory-motor networks. A pair of nonspiking neurons in the leech nervous system, the NS cells, is present in each midbody ganglion and displays a very extensive arborization. These neurons are electrically coupled to virtually every excitatory motoneuron, playing a role in the regulation of their coactivity, which may be important for the coordination of motor activity. In addition, NS neurons are part of the interneuronal layer that mediates between mechanosensory and motor neurons. Given the wide influence of these neurons on effector neurons, it has been of interest to analyze how sensory input is integrated by the NS neurons. In spite of the fact that NS neurons do not display Na+-dependent spikes, under certain circumstances they fire low threshold spikes (LTS) that reveal that these neurons exhibit voltage-dependent-Ca++-conductances (VCC). Using a variety of fluorescent calcium probes we observed that these LTS caused a widespread increase in Ca++ that rose with no measurable delay throughout the entire arborization of the NS neuron. To learn whether VCCs in NS cells were also activated by synaptic input, mechanosensory P cells were stimulated to fire 5 spikes at 8-25 Hz. This evokes responses that combine depolarizing and hyperpolarizing phases. We observed that depolarizing synaptic responses evoked Ca++ transient, whose magnitude correlated with the magnitude of the synaptic potentials. Upon strong depolarizing responses the Ca++ signals were observed in the four primary branches and the principal trunk of the NS neuron. Ipsilateral and contralateral stimulation seemed to evoke a different spatial distribution of Ca++ signals. The results suggest that VCC play a role in the propagation of synaptic responses throughout the branches of the nonspiking neuron.

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P 366

Head, body and wing kinematics of budgerigars (Melopsittacus undulatus) executing complex flight maneuvers

We investigated the kinematics of the body and the head of budgerigars during flight through a tunnel. The tunnel presented an aperture consisting of a vertical slit, 17 cm wide, positioned halfway along its length. The slit extended from the floor to the ceiling and was flanked by two soft cloth walls, to prevent injury. High-speed stereo cameras were used to film and reconstruct the flights. Diamond-shaped tracking dots were affixed to the head and the body to track their yaw, pitch and roll movements. The wing tips were also tracked. In control experiments without obstacles, the head and body maintained a horizontal orientation and the wings executed flapping motions throughout the flight, which was through the middle of the tunnel. In contrast, when flying through an aperture, the birds approached the aperture either from the left or the right (depending upon the individual) and executed a sharply banked turn prior to entering the aperture. The roll was clockwise or counterclockwise, depending upon whether the bird entered the aperture from the left side or the right. When passing through the aperture the wings were either held closed, or stretched outwards (i.e. upwards and downwards from the banked body) to enable collision-free flight. Presumably, the roll facilitates safe flight through the aperture when the wings are extended, and approaching the aperture from the left or right pre-compensates for the lateral movement of the bird that would unavoidably accompany the roll. Flight through a narrow passage appears to be an intricately pre-planned exercise. In contrast to the body, the head exhibited relatively little roll or pitch during the entire maneuver. Evidently, it is important for the head to maintain a stable horizontal attitude during these maneuvers, despite the large roll movements of the body. Indeed, mechanically clamping the body to the head disrupts flight in pigeons [1]. [1] Warrick et al. (2002) Am. Zool. 42:141-148

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P 367

Hide and Seek in the common chameleon¡¦s (Chamaeleo chameleon) avoidance responses: Visuo-motor patterns, lateralization and ontogeny.

Common chameleons (C. chameleon, Reptilia, Lacertilia) are arboreal lizards. Their optical nerves show full decussation, and visual information from each eye is conveyed to, and processed by, the contra-lateral optic tectum with minimal tectal connection. Chameleons have highly independent eye movements, allowing them to rapidly shift from monocular to binocular vision. From hatching, faced with a threat stimulus, a chameleon positions itself on the side of a perch opposite (ƒî180¢X) to the stimulus, while keeping it under monocular or binocular viewing. Sideways motion of the stimulus results in the chameleon"s counter-motion by the chameleon. Aims: Using the visuo-motor patterns of avoidance response as a model, to determine, under conditions of binocular and monocular viewing, (i) the visuo-motor patterns used, (ii) evidence for lateralization, and (iii) ontogenetic changes. Methods: Chameleons (n=25), were subjected to an apparently moving threat, by the slow rotation of their vertical perching pole. Pole motion was clockwise or anti-clockwise in discrete steps, relative to the stationary experimenter. Chameleons of 4 age groups (1, 120, 240, 360 days post hatching) were tested on a narrow or a wide pole, respectively allowing monocular and binocular viewing or only monocular viewing of the threat. Video films were computer analyzed for response parameters (amplitude, latency). Results: (i) The proportion of individuals showing avoidance response increased significantly with age, (ii) The majority of motor parameters analyzed showed significant ontogenetic changes, (iii) Within age groups, the directionality of threat approach had a no apparent effect on the response, (iv) However, individual differences were found as some showed clear laterality of response. The results indicate that, in the Common chameleon, laterality may occur in certain aspects of the visuo-motor avoidance response, and may well be an individual but not a population trait.

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P 368

Motor planning modulates sensory-motor control of frog collision avoidance behavior.

Our behavioral experiments by using computer graphics simulating an approaching object combined with frame by frame analysis showed that the frog produces collision avoidance behavior when the retinal size of the object reaches a threshold value (Yamamoto et al., Brain Behav. Evol. 62, 201-211). In this study, we closely examined the effect of stimulus location in the horizontal plane on frog collision avoidance behavior by measuring stimulus position relative to the animal, threshold angular size and angular velocity of escape behavior. We found that the animal performed □gcut-back□h turn toward the stimulus presented in the upper visual field. The correlation analysis between angular velocity of escape behavior and stimulus location or escape turn angle across whole visual filed showed that the velocity was more strongly correlated to escape turn angle (r=0.758) than stimulus location (r=0.635). This suggests that the frog controls angular velocity of avoidance behavior on the basis of its own motor planning rather than stimulus location. Further examination showed that the frog changed the escape strategy depending on the visual field. Within a zone extending 60 degrees on either side of the midline, the escape velocity was accurately controlled to make behavioral time constant (about 250ms), while the threshold size quite varied. The escape velocity and the threshold were not correlated significantly (r=0.081). On the other hand, within the visual field more than 60 degrees from the midline, the escape velocity was not controlled accurately due to large turn angle and behavioral time varied. The escape velocity and the threshold were positively correlated (r=0.382). This suggests that the threshold is controlled to compensate long lasting behavioral time due to large turn angle and unsuccessful control of velocity within the caudal visual field. Altogether we concluded that the frog controls both angular velocity of avoidance behavior and threshold angular size on the basis of its own motor planning for prospective behavior.

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P 369

Head motion of the barn owl (Tyto alba) during natural visual search

Barn owls use stereovision to detect small prey in dimly lit conditions. In barn owls the eyes are largely fixed in the skull, consequently, saccades to objects of interest are performed by head movements. However, in addition to the rapid head saccades barn owls exhibit a variety of other stereotypic head movements. The function of these movements in vision is unknown. To investigate these movements we measured head kinematics in perching, freely moving barn owls. Head motion tracking is achieved via the ViconTM motion capture system. We have designed a special head mounted device that is attached to the owl's head (total weight 17gr). The tracking device is equipped with five reflectors tracked by the Vicon system, a miniature wireless CCD camera and a battery pack for the CCD camera. The CCD camera is fixed and is oriented along the visual axis of the owl. Motion measurements of the markers are carried out at sub mm resolution and are performed at 120 frames per second. The kinematic measurements are combined and synchronized with video images recorded from the miniature head-mounted camera. This setting allows us to measure detailed kinematics of head motion while simultaneously analyzing the visual scene and recovering the fixation point. In the first part of this research we have isolated and characterized stereotypic side-to-side head motions. We used screw theory to describe the instantaneous head motion of the owl. The screw coordinates provide us with information on the nature of the head motion, i.e. pure rotation/ pure translation or a helical motion. The coordinates also provide information on the instantaneous biomechanical axis of the head motion. This data is analyzed to provide information and understanding on the kinematic nature of the owl head motion and is also used for clustering typical head motion behavior of the owl. This work is supported by a grant from the German Research Foundation.

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P 370

Gaze following and anticipation in the scanpaths of monkeys viewing videos with social content.

Visual scanpaths were recorded from 3 adult male monkeys during the presentation of movies clips depicting conspecific social displays. The monkeys in the movies displayed appeasing (lipsmack), neutral, or aggressive (threat) postures, gestures, and facial expressions. Each 10-s clip contained multiple repetitions of the same type of display, directed both at the viewer and at recipients not visible in the movie frame. The target of the gaze of the viewer monkeys was localized in each frame and its location was coded by an ethogram that contained rubrics for the most common areas that attracted the gaze of the viewer (e.g., body parts, face areas). Gaze following was coded when the viewer monkey was looking at the eyes of the movie monkey and made saccades in the direction of the gaze of the display monkey. Monkeys Q, H, and T, viewed 60, 54 and 53 videos respectively. Gaze following was observed in, 28%, 53%, and 33% of the movies for monkeys Q, H, And T respectively. Gaze following was initiated in all three viewers by the same video segments. Threatening facial expressions were more likely to elicit gaze following. Anticipation was coded as saccades that preceded the direction of action/display/gaze of the display monkey by at least one frame. Although the monkeys habituated to the repeated presentation of the movies, they showed, on occasion, spontaneous anticipation (2, 15, and 3 observations for monkeys Q, H, and T respectively). The earliest trial when anticipation was recorded was the 4th, 3rd, and 9th trials for monkeys Q, H, and T respectively) Spontaneous gaze following and anticipation in monkeys indicate shared mechanisms of social cognition with humans. The large overlap in the elements of the movie that elicited gaze following in all three monkeys indicates the existence of species-specific behavioral triggers for gaze following.

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P 371

Salamander tongue projection is based on target motion extrapolation

For a predator, successful prey capture is a matter of life or death, and the neural circuits driving it should be robust to variations in hunting conditions. To learn how these conditions influence the behavioral strategy, we placed a freely moving salamander (eurycea) and a freely moving fruit fly (drosophila) in an arena and quantified 272 high speed video clips of tongue projections over a 5 log-unit range of light intensities, and a wide range of target positions, sizes and speeds. Capture success was 60% in scotopic light (0.005 µW/cm2), peaked at 90% in mid-photopic light (10 µW/cm2) and fell to 70% in high photopic light (200 µW/cm2). Tongue projection distance varied linearly with target distance over a 4 to 27 mm range and the projection depth error was small (1.7 ± 2.5 mm). Tongue projections were ballistic and had the same acceleration and velocity profile independent of distance. Angular error was greater for nearby flies than for distant flies. That these errors decreased with target distance suggests they are predominantly neural, rather than kinematic in origin. The salamander maintains a two-tongue width error margin independent of target distance, allowing a sufficient safety margin to ensure capture success. Many tongue projections (61%) were preceded by a rapid head rotation. We found that an extrapolation model (in which the tongue is launched to the location the fly is moving towards when the head turn begins) predicted actual projection angles two-fold more accurately than a model which projects to the location of the fly prior to the head turn. The salamander appears to estimate both position and velocity of its target in a window 100-400ms before tongue projection, and uses this information to make a precise head turn culminating in a launch of the tongue. Projection errors increased when the fly accelerated during the head turn, suggesting that the extrapolated fly position is not strongly modified by visual feedback during the head turn.

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P 372

Ultrasonic gymnastics: Motor control and neural processing constraints of echolocation in bats

Bats commonly use echolocation to locate, characterize and track objects in their immediate surroundings. Echolocation is the use of an active spatial sensory system where the individual produces ultrasonic calls and extracts auditory cues about the environment from the returning echoes. Prey capture is characterized by an increased rate of calling, culminating in a rapid buzz phase during which bats produce calls that sweep from about 55 kHz to 20 kHz in 1 ms up to 160 times per second. Such rates push the envelope of both sound production and neural processing performance. However, which of the two limits auditory information streaming from the environment remains an outstanding question. We used a multi-microphone array in a flight-room to measure aerial attacks in Daubentons' bat (Myotis daubentonii). Using this data we reconstructed the 3D flight path of the bat as well as its call directionality. Also, using new computational methods, we were able to reconstruct the echo signal as received by the bats to determine how calls overlay with echoes and as such confound neural processing. In addition, we measured the in vitro performance of the main frequency modulating vocal muscles using a specialized workloop setup for superfast muscles. We measure that these muscles produce positive work up to 160 Hz in vitro and therefore establish the first case of superfast muscles in mammals.

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Modulation of hippocampal activity by dynamic cues

Tracking of dynamic cues is an essential function for animals' brains, since it is critical for activities such as hunting, successful foraging, flight from predators or mating. This study is focused on the role that dynamic cues play in the modulation of brain activity. For that purpose, the EEG in the rat hippocampus was recorded and the theta band, 4-12 Hz, was then analyzed. It is known that the theta band is associated to the speed of the rat as well as to several cognitive functions (Buzsáki, 2005). Despite the fact that the tracking of moving objects has been profusely studied in various animal species (Fattori et al., 2009),little attention has been paid to it in rodents and the few existing studies used images presented on a screen (Klement et al, 2010) and not real moving objects. On the other hand, the hippocampal system coding for the animal's self-tracking (place cells) has been thoroughly studied in rodents (O'Keefe and Nadel, 1978). Our objective here was to understand the neurological basis of the tracking system for moving objects in rodents, and its interactions to the self-tracking system. We chose a robot (e-puck, EPFL) as the dynamic cue and the animals were trained to track its movements in order to receive reward. When animals achieved stable performance of 80-90 % of correct choices were chronically implanted and EEG was recorded. In order to avoid the modulation of theta by the self-movement animals were trained to do the same motor task without the robot; the robot was substituted by a sound that triggered the onset of the trial. Preliminary data shows a difference in the theta activity between the protocols with and without robot. While during the protocols involving the robot the power of the theta band increased after the onset of the stimulus, this increase did not occur in the absence of the robot. That difference suggests that the movement of the robot effectively induces synchronization in the theta band.

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Vision II

486 **P 374**

Behavioral Roles of Individual Cell Types in the Fly Early Visual System

Brains consist of anatomically and genetically distinct classes of neurons. An important question is what function individual cell types play in shaping complex behaviors. We are attempting to discover the behavioral roles of individual cell types in the early visual system of the fly, Drosophila Melanogaster. The layer of the fly visual system immediately postsynaptic to the photoreceptors, the lamina, consists of less than a dozen identifiable cell types- we use the Gal4-UAS system and genetic intersectional strategies to express blockers of synaptic transmission in each of these cell types. We then tested flies within a virtual reality flight simulator for defects in visually-guided flight behaviors. Blocking synaptic output simultaneously in the four primary cell types of the lamina (L1-L4) renders flies blind to visual motion stimuli. However, silencing each cell class individually does not abolish the optomotor response, a classic test for motion sensitivity in the fly. More specific and targeted behavioral assays reveal specialized roles for some individual classes of lamina neurons. Our results suggest that individual cell types of the fly visual system perform overlapping roles in some general visual behaviors, but specific deficits are measureable under certain behavioral conditions.

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P 375

EFFECT OF CENTRAL PROCESSING ON VISUAL MOTION RESPONSES IN DROSOPHILA

Attention-like processes modulate behavioural responses to visual stimulation. Here, we use the Drosophila model to characterize behaviour to visual stimuli and dissect its neural correlates. We developed a novel high-throughput paradigm that scores responsiveness of fruit flies to a variety of visual stimuli in under 10 minutes. Flies were exposed to visual stimuli while progressing through mazes overlaid on top of CRT monitors. Upon completion of the maze, flies emerged at the exit points and were automatically counted, after which an average response, the optomotor index, was calculated for each experiment. Wild-type flies and dunce1, a learning and memory mutant, were used to evaluate this automated paradigm. Previous work has shown that dunce1 flies display an increased visual response compared to wild type (Canton S), and this has been attributed to defects in visual suppression relevant to selective attention [1]. dunce1 mutants responded more strongly than wild type flies to moving stimuli across a wide range of luminosity, contrast, spatial, and temporal frequency values ($p<0.05$, $n=192$). To further dissect visual motion responses in dunce1 mutants, we introduced conflicting visual stimuli. dunce1 mutants retained a higher response than wild-type flies even when competing stimuli were occluding most of the field of view ($p<0.05$, $n=202$). Since dunce is strongly expressed in the mushroom bodies, the learning and memory brain structures, we hypothesized that they are involved in producing these optomotor responses. Expressing wild-type dunce cDNA in the mushroom bodies rescued the visual response to wild-type levels. Conversely, silencing the synaptic output from the mushroom bodies produced dunce-like visual responses. We have developed an efficient new method of screening visual phenotypes in Drosophila, and our results suggest that the mushroom bodies, structures previously associated with olfactory learning memory, are also involved in modulating visually driven behaviour in flies. 1. van Swinderen B, Flores KA (2006) Attention-like processes underlying optomotor performance in a Drosophila choice maze. Dev Neurobiol. 67:129-45.

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P 376

Identifying synaptic circuits in the fly's medulla column from ssEM

Synaptic circuits in the Drosophila brain now begin to yield to new methods using serial-section EM (ssEM). The medulla of each optic lobe is the largest brain neuropil with > 60 morphological cell types, but is: a) arranged in ~750 columns with a repeating structure of input elements from the first neuropil, the lamina, where the anatomical circuits are well identified; b) layered in 10 strata respected by both the input terminals of lamina neurons and the arbors of morphologically determinate medulla cells. We identify these from previous Golgi impregnation reports, or recent data from genetic reporters using the Gal4/UAS system. From a series of 40-nm EM sections, a 100 x 100 µm area was imaged and aligned in ~1800 sections, from which we have reconstructed cells within an area ~9 x 9 µm covering a single column through its 50 µm depth. In this volume we identify many columnar cells, with axons that run down the column axis. These include terminals from: lamina cells L1-L5, photoreceptors R7 and R8, and centrifugal cells C2 and C3. Synapses of R7/R8 are mostly triads, while those of L1 and L2 are mostly tetrads. Shapes of the medulla neurons conform closely to Golgi and GFP labeled profiles, providing assurance that our reconstructions are complete and accurate. We have identified medulla targets for lamina pathways, as follows. a) Spectral pathways from R7 and R8: amacrine neuron Dm8, which receives pooled R7 input; Tm5; and Tm9 (Gao et al., Neuron 2008). b) L1 and L2 with crucial roles in motion detection, for L2: Tm1 and Tm2, frequent partners at postsynaptic triads; and for L1: Mi1 and other neurons (L5, C2, C3, Tm3). L5 connects reciprocally to L1 and L2. Most synaptic contacts are redundant and connect each terminal sparsely with few medulla target cells. ssEM reconstructions, still a lengthy if accelerating process, will be essential for later functional identification by genetic dissection methods. Support: NIH EY-03592 (to I.A.M.); and HHMI.

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Visual Selective Attention in Drosophila melanogaster

In a cluttered visual environment, not all parts of the visual field are processed at any time. The most relevant information is selected and the rest is filtered out. Visual selective attention has been demonstrated in Drosophila earlier. It can be directed through external visual and odorous cues. Recently, we have established robust conditions for studying various characteristics of the externally directed visual selective attention in Drosophila during tethered flight. Using these conditions we have been able to orient the fly's attention to one or the other visual half-field through attractive or aversive visual cues. We have discovered that the orienting cue can precede the test temporally up to a few seconds and that it can be spatially separated from the test stimulus and yet orient the attention. We have also observed that this kind of external orientation of the attention seemingly works only in the visual field below the horizon for the fly. The mushroom bodies don't seem to be essential for this kind of attention.

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P 378

Citius, Fortius, Altius? The faster a fly walks, the stronger its motion-vision neurons respond to higher image speeds

Changes in behavioral state modify neural activity in many animals. Here we report state-dependent changes in neuronal tuning during walking in the visual-motor pathway of Drosophila. We used two-photon imaging and a genetically encoded calcium indicator, GCaMP3.0, to monitor intracellular calcium activity in motion-sensitive lobula plate tangential cells (LPTCs) in head-fixed Drosophila walking on an air-supported ball. The ball's motion is tracked at 4 KHz and can be used as a proxy for the fly's own movements. When presented with horizontally moving visual patterns, flies displayed robust compensatory turning on the ball. Neurons of the horizontal system (HS)—a subgroup of LPTCs thought to be involved in optomotor behavior—responded with strong calcium transients correlated with the simultaneously recorded angular rotation of the fly [1]. HS neurons displayed stronger calcium transients in response to motion stimuli when flies were walking rather than resting [2], and the strength of their responses was correlated with walking speed. Moreover, HS neurons showed a greater response gain for higher temporal frequency motion stimuli, shifting their temporal frequency optimum towards higher speeds. When an animal moves through its environment, its retina is exposed to higher image speeds due to self-motion. Thus, walking-dependent modulation of HS neuron tuning in the Drosophila visual system may constitute a mechanism to facilitate processing of faster retinal image shifts in behavioral contexts where these speeds of visual motion are relevant for course stabilization. More generally, our preparation enables physiology in tethered walking and flying Drosophila, and provides a platform for future explorations of decision-making and sensory-motor integration in the central brain of this powerful genetic model organism. 1. Seelig, J. et al. Nature Methods (2010). 2. Chiappe, M.E. et al. Current Biology (in press).

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How blowflies analyse the motion of visual patterns

Flying animals require robust stabilization reflexes even when facing ambiguous sensory inputs. Visual information indicating changes in flight attitude, for instance, is based on self-motion-induced optic flow but may be masked by motion of visual contrasts in the surroundings such as foliage moving in the wind. The blowfly is an excellent model system to study how this problem may be solved. Its optomotor response is well-characterised, as are its optic flow-processing interneurons supporting flight and gaze stabilization, the lobula plate tangential cells (LPTCs). To find out how blowflies integrate conflicting motion cues, we presented them with “plaid” stimuli, formed from pairs of superimposed gratings moving in divergent directions, while measuring their yaw torque response. Surprisingly, the response was not tuned to the overall direction of pattern motion, contrary to current models of local motion integration in superposition eyes. Instead, the response was tuned to a complex function of the plaid components. To compare behavioural outputs with neuronal signals we recoded from spiking LPTCs while presenting flies with plaid stimuli. H1 and V2 LPTCs were also tuned to a complex function of the plaid components. These cells integrate signals from directional elementary movement detectors (EMDs) across the visual field. We could only model the cells’ responses by assuming two EMD properties: 1) a novel, non-cosine directional tuning function and 2) a saturating output non-linearity. In contrast, the V1 cell, which integrates signals from LPTCs VS1-3, was tuned only to the direction of pattern motion. To accurately model V1 cell activity we assumed VS1-3 response properties to be the same as those of the V2-cell, except for their respective directional preferences. Our results suggest that blowflies employ more than one strategy for coping with conflicting visual motion cues, and extend current theories of local motion integration in superposition eyes.

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P 380

Computation of sensitivity for motion and directional selectivity in the blowfly visual system

The detection and the neural processing of motion is an essential task of the visual system. Although the properties of visual motion-sensitive neurons in vertebrate and invertebrate brains have been studied to some detail, the cellular mechanisms leading to direction-selective responses to motion remained largely unresolved, because the input circuitry of these neurons is often difficult to access with electrophysiological techniques. Much of our knowledge on motion computation stems from recordings of the axonal output signals of lobula-plate tangential cells (LPTCs) in the fly brain. These neurons have large receptive fields and some of them show a pronounced specificity for distinct patterns of optic flow, i.e. retinal image shifts during self-motion. However, at this time it is not precisely known how these neurons obtain their sensitivity for motion in distinct directions, because the small size of their retinotopically arranged local input elements renders electrophysiological recording an inefficient approach. We circumvented this problem by monitoring localized neuronal activity in two types of calcium imaging experiments. 1) By measuring the spatial patterns of calcium concentration changes across the large dendrites of LPTCs we visualized regional activity elicited by local presynaptic neurons providing retinotopic input to LPTCs. 2) We stained small groups of neurons in the medulla, the input neuropile to the lobula plate, with calcium-sensitive dyes via local electroporation. We found that calcium signals at LPTC dendrites are highly direction selective, with characteristic spatial variations of the local preferred direction across the dendrite. The recordings in the medulla show that motion sensitivity is already expressed at this stage of the visual pathway. However, motion responses in the stained area of this tissue appeared to be uniformly omnidirectional, with no clear localized preference for single motion directions discernible so far.

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P 381

Effect of light intensity and wavelength on take-off orientation in Rhodnius prolixus (Reduviidae: Triatominae)

Chagas disease is a chronic parasitic infection caused by Trypanosoma cruzi and endemic in 21 Latin-American countries. Transmission to humans is made by insects of the subfamily Triatominae. In Colombia, Rhodnius prolixus (Stål, 1859) is the main vector due to its broad geographical distribution, high frequency of dispersion and tendency to have large populations in human dwellings. The arrival of these insects from natural habitats (tree palms) to human dwellings seems to occur throughout flights in adults. Under natural and laboratory conditions R. prolixus is highly attracted to incandescent lights. Having this in mind we decided to evaluate in laboratory the take-off orientation in R. prolixus to combinations of different wavelengths and light intensities. A 2 m3 tent of white semitransparent cloth with a take-off platform divided in eight equal sectors was used as experimental arena. Light sources with six different wavelengths (370, 450, 520, 590, 623 and 660 nm) and three intensities (6, 0.6, 0.06 µW/cm2) were used as attractive stimuli. Five females and five males were tested per night at each wavelength and intensity in twelve nights (18:00 to 6:00 h, 21-27 °C and 33-50 % relative humidity). Each night the orientation of take-offs was recorded with an infrared video camera and analyzed with circular statistics. Differences in take-off between genders were compared by means of goodness of fit, Chi-square tests. A relationship between gender and take-off in R. prolixus was not found. Highly attractive wavelengths were 450, 520 and 660 nm. However, the role of each wavelength on attraction changed depending on intensity of the light source. Red lights attracted more R. prolixus than blue and green lights at lower intensities.

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P 382

Active gaze strategies in two fly species

In normal life motion vision is always performed under closed loop conditions. The way an animal moves shapes its visual information, which is then analysed and used for motion control. Different movement hence change visual information availabilty. Flies are known to segregate their movements in to (a) fast rotations of short duration, termed saccades, and (b) intersaccadic intervals of translatory movements and variable duration. This is believed to facilitate the perception of the three-dimensional layout of the environment. The two fly species Calliphora vicinia and Eristalis tenax have homologous motion sensitive visual interneurons, but exhibit different flight styles. We compared both species' potential neuronal adaptations to the different motion dynamics resulting from these flight styles. To reduce the enormous complexity of the performed flight manoeuvres, we employed clustering algorithms to derive a small set of prototypical movements (PMs). This allows us to segregate the seemingly continuous movements that constitute an animals flight trajectory into a limited number of components, called prototypical movements (PMs). From the transition probabilities between the different PMs we derive a set of rules, i.e. a kind of syntax of the behaviour. As a consequence of the closed-loop nature of the action-perception cycle, each PM leads to characteristic visual input changes. We determined these image shifts on the retina by reconstructing the flight trajectory and arena in virtual reality and by rendering the correpsonding distinct optic flow patterns. Even though both species exhibit a similar saccadic flight style, we found the visual input to vary greatly between fly species. In a constrained environment, as for example a small flight arena, Calliphora flies at nearly ten times the speed of Eristalis. In contrast to Calliphora which mainly flies forward, Eristalis moves at slow speeds in nearly every possible direction. However, in open space Eristalis reaches similar flight velocities to Calliphora. To test whether identified homologous visual interneurons are adapted to the specific optic flow encountered by the two fly species, respectively, we presented the reconstructed behaviourally generated optic flow of both species on a high-speed surround LED stimulator (FliMax) to individuals of either species. By using the segregation of flight trajectories into sequences of PMs, we characterised the neuronal response patterns to distinct movements components. The neurons in both species responded similarly to flights of both species, although the response amplitudes of Eristalis neurons were larger under both stimulus conditions than Calliphora. Currently we are analysing response patterns to pinpoint specific adaptations and their advantages.

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P 383

Multi-sensory control of flower inspection movements in a nocturnal and a diurnal hawkmoth

A crucial stage in the interaction between pollinators and plants is the moment of physical contact between them, known as flower inspection. Nectar guides â€“ conspicuous colour markings of flowers â€“ have been shown to influence the inspecting behaviour in many insects, particularly in diurnal species. For the nocturnal hawkmoth Manduca sexta tactile information has an important role in flower inspection, but there is no knowledge about the use of visual nectar guides in this behaviour. Here, we carried out a series of experiments to first, evaluate the putative visual control during flower inspection and second, to explore how simultaneous tactile and visual guides could influence this behaviour. Results show that some visual guides could â€˜leadâ€™ probing attempts towards the nectary, improving inspection efficiency relative to plain coloured controls, while others "mislead" the probing behaviour, decreasing chances of finding the nectar rewards. We also recorded inspection times, finding that moths can learn to inspect flowers more efficiently when visual guides are available. When flower models offered conflicting tactile and visual information, visual guides influenced proboscis placement, while tactile information controlled proboscis movements. These results show that innate inspection behaviour is under multimodal sensory control, consistent with other components of the foraging task. We discuss results in relation to the impact that these responses have in the interaction that M. sexta has with their natural guild of flowers. Additionally, we present results from similar experiments performed in the diurnal hawkmoth Macroglossum stellatarum, and discuss differences in sensory control of behavioural responses in the context of their different ecology and monophyletic history.

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Honeybee landing: is the final phase guided by stereo vision?

A honeybee approaching a flower relies primarily on vision to judge the distance to the flower, and to reduce its speed appropriately and accurately to perform a soft landing. The landing insect has access to a repertoire of visual cues based on optic flow, such as motion parallax and image expansion, to control its approach. Honeybees use these cues to reduce their speed when approaching a horizontal surface (1). However, the final phase of the landing involves a hover prior to touchdown (2). During this phase, cues derived from optic flow would be weak or nonexistent. One possibility for the bees to gauge the distance to a surface during this phase would be to use binocular disparity cues. The theoretical range over which honeybees can employ stereo vision is limited, but the observed hovering phase falls within this range (2). To investigate whether honeybees use stereo vision for close range tasks, we have filmed and analyzed the behaviour of bees during the final moments of landing. Bees were trained by food reward to land on a platform, titled at various angles. One group of bees was then caught, and one of the eyes was occluded using semi-opaque nail varnish. The landing behaviour of such monocular bees was filmed, and compared with that of a group of untreated honeybees. The untreated individuals displayed a consistent landing behaviour with a reduction of speed, followed by a hover phase before extending their legs. However, the monocular bees did not reduce their speed as markedly, and did not hover prior landing. They often collided with the platform, making first contact with their heads. Monocular bees also displayed larger lateral movements during the final approach. These results indicate that honeybees may utilise stereo vision to guide the final moments of landing, when the substrate is sufficiently close to permit reliable range information to be extracted. References [1] Srinivasan et al. (2000) Biol. Cybernetics 83, 171-183. [2] Evangelista et al. (2010) J. Exp. Biol. 213, 262-270.

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Visual processing in the honeybee brain: optophysiological recordings of the lobula-anterior optic tubercle tract

In insects, visual information processed in the retina is conveyed to the optic lobes of the brain, consisting of the lamina, the medulla and the lobula complex, where it is processed in a hierarchical manner. From there, it is sent to various protocerebrum regions, which can be roughly divided into dorsal and ventral regions. Dorsal foci are known to process optic- flow cues over large visual fields, thus contributing to the control of flight posture and probably to distance estimation. Functional studies on ventral foci, on the other hand, are scarce. Here we focused on a subset of neurons connecting the lobula with one of these structures, the anterior optic tubercle (aot) in the honeybee, Apis mellifera, an insect reputed for the sophistication of its visually-mediated behaviors. To study lobula-aot neurons, we developed a preparation that allows visual stimulation of the bee compound eye with simultaneous access to the visual neuropiles of the honeybee brain for optophysiological calcium imaging recordings of population activity. Achromatic/chromatic stationary and motion stimuli were presented to the lateral region of the bee□fs compound eye using a LCD monitor, ipsilateral to the measuring side. Lobula-aot neurons reliably responded to these stimuli. We found 1) distinct activations to the presentation of a white rectangle on different eye regions; the difference in response patterns was larger between dorsal and ventral eye regions than between anterior and posterior eye regions; 2) specific and distinct activations for blue and green lights, and a reduction in activation upon stimulation with a mixture of blue and green; such a non-linear response is in agreement with color-opponency phenomena, and 3) evidence for motion-direction coding without directional polarity. Our results thus show that lobula-aot neurons participate in the processing of object spatial location, color and movement.

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P 386

Anatomical and functional optical imaging study of visual processing in the anterior optic tubercle of honeybees

Color vision in honeybees has been intensively studied at the behavioral level and with a number of intracellular electrophysiological recordings allowing the study of one particular neuron at a time. However, our knowledge of visual processing in bees is still limited because no technique has yet allowed the functional study of visual processing at the circuit level. We present such an endeavor in a little-studied visual area of the bee brain, the anterior optic tubercle (AOT). We characterized the neural connectivity and the role of the AOT in visual processing, using two main approaches: 1) neuro-anatomical characterization of visual pathways to and from the AOT, and 2) calcium imaging of visual stimulus-induced signals in the AOT. We show that the AOT receives visual input from different regions of the lobula and that this input is segregated in the AOT, which is compartmentalized into two main subunits. Each AOT provides visual input to the contralateral AOT through two distinct tracts. For calcium imaging, the AOT was retrogradely stained with a dextran-conjugated calcium indicator. Our experiments based on stimulation with LED arrays show that: 1) Lights presented in different visual fields induce distinct patterns of neuronal activity in the AOT, especially for dorsal and ventral eye regions. These and the anatomical results suggest that retinotopic organization is retained in the AOT; 2) Monochromatic light of different wavelengths matched to the bees' photoreceptors (UV, blue and green) induced different signal intensities and activity maps, showing a spatial coding of chromatic stimuli in the AOT; 3) Activation by blue-green polychromatic stimuli was always lower than to the strongest component (green light), revealing the existence of inhibition phenomena ('suppression'). Our results strongly suggest the involvement of the AOT in retinotopic processing of chromatic information in the bee brain.

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P 387

Sticking the head into the wind: The effect of wind and the role of vision on the landing performance of honey bees

In their natural environment, animals are exposed to a variety of different conditions. Their efficiency in handling changing environmental conditions depends primarily upon how well their sensory perception and motor control have adapted in the evolutionary process. Flying requires the most sophistication in sensory and motor capabilities. In particular, a controlled landing under windy conditions is one of the most challenging tasks that an insect encounters frequently. In this study we investigated the effect of wind on the landing behaviour of bees, and the role that vision plays in this context. Bees were trained to enter an experimental chamber and land at a hole, in the centre of a platform. The platform was covered by different visual patterns. A laminar airflow was created over the platform. Body orientations and movement trajectories of the bees were filmed and reconstructed in 3-D using two high-speed cameras. Analysis of the trajectories revealed that, under no-wind conditions, the bees tend to move towards the target hole, regardless of their position relative to it. They also tended to point their long axis towards the target. On the other hand, in the presence of wind, body orientation and flight direction tended to be oriented into the wind. Thus, when there was wind, bees displayed a strong tendency to land into the wind. Unexpectedly, bees flew faster in the presence of wind and flew more directly towards the target. Bees do not appear to rely solely on the visual texture to sense and prevent drift, but the visual contours can expedite or prolong the landing process. The most 'natural' pattern ('sector') provided the best guidance, whereas the 'random', 'ring' and 'lines perpendicular to wind' patterns elicited slow and tortuous trajectories. Interestingly, the 'blank' pattern, which should provide the least visual guidance, did not seem to hinder the approach, raising the question what other sensory systems could be playing a role.

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The LGMD Model Mean Response as a means to a robot vision system for Collision Avoidance and Target Following

The Lobula Giant Movement Detector (LGMD) neuron responds selectively to rapidly approaching objects. This fact and the simplicity of associated equivalent models make the implementation of the LGMD model in mobile robotic systems attractive. In this article, we are interested in understanding the LGMD model previously proposed by (Yue, et al., 2006) and the achieved properties of the model versus the ones of the real neuron. The model is extensively studied when submitted to relevant simulated visual data sets. These studies enable us to understand some of the model limitations and propose a new methodology to cope with them for obstacles detection and avoidance (Guest and Gray, 2006). Additionally, we propose some model changes to achieve target following, demonstrated in a mobile robot able to follow another robot. The system performance is verified in two distinct experiments in which a mobile robot equipped with a camera on its top, must autonomously navigate in a simulated environment. In the former, the robot is surrounded by obstacles and the obstacle detection algorithm is only based in the LGMD model implemented in the robot. In the second, the robot must follow another robot, which can increase or decrease its forward velocity or move laterally. The obtained results show the system robustness and reliability.

Guest, B.B. and Gray J.R. (2006). Responses of a looming-sensitive neuron to compound and paired object approaches. J Neurophysio,l 95, pp. 1428–1441. Yue, S., Rind, F.C., Keil, M.S., Cuadri, J. and Stafford, R. (2006). A bio-inspired visual collision detection mechanism for cars: Optimisation of a model of a locust neuron to a novel environment. Neurocomputing, 69, pp.1561-1598.

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P 389

Role of HCN channels in neural computation of a looming sensitive neuron

Integral to collision avoidance and escape behavior in the locust, Schistocerca americana, is the lobula giant movement detector neuron (LGMD). The LGMD is a looming-sensitive, interneuron in the optic lobe that was previously shown to compute angular threshold and maintain receptive field invariance across a wide visual angle, which both require nonlinear computations. This project uses this model system to examine the role of the hyperpolarization-activated inward rectifying current (Ih) in neural computation within a well-defined behavioral context. Application of the Ih blocker ZD7288 caused decreased response to looming stimuli to levels below those found previously to initiate escape behavior. Ih blockage additionally removed inward rectification, increased input resistance from 4 to 10 MΩ on average, and hyperpolarized the resting membrane potential (RMP) by 2-12 mV. Hyperpolarizing pulses produced a depolarizing sag which increased linearly with the amount of hyperpolarization below RMP. The time constant, τ, of the sag and post stimulus rebound also depended on membrane voltage, and suggests an average τ=600-800 ms for the h channels at RMP. However, repeated strong hyperpolarizing pulses suggested a more prolonged opening of the conductance, with τ>1 s. Impedance profiles were calculated from the responses to chirp stimuli. The impedance amplitude depended strongly on the membrane potential, revealing the effects of the h current. However, unlike previous investigations, Ih had no apparent effect on the impedance phase in the LGMD. Consistent with these findings, blockage of Ih by ZD7288 produced a large increase in low frequency impedance amplitudes, but had no effect on the impedance phase. The h channels were electrophysiologically characterized at multiple points within the large dendritic arbor of the LGMD to investigate their role in the computations underlying collision avoidance behaviors.

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P 390

Robustness of coding in ocellar neurons of the locust, Schistocerca gregaria.

A locust has three ocelli that collect light over a wide visual field, and one of their functions is to stabilise flight attitude with reference to the visual horizon. This is achieved by connections from ocellar 'DN' neurons that receive multimodal sensory inputs in the brain and make connections in the thoracic ganglia with flight motor neurons and interneurons. Previously, I have found that one DN neuron, DNI, uses a relatively sparse spike code to signal fluctuations in light, but times individual spikes to sub-millisecond precision. Rather than signal slow changes in horizon position, this coding strategy suggest that DNI provides information during each wingbeat to advance or delay timing of contraction of particular muscles. Here I investigate whether this coding strategy is sufficiently robust to persist under changes in two different behaviourally significant conditions. First, it is possible other sensory modalities would mask the way DNI encodes light stimuli into spikes. DNI is excited by wind-sensitive hairs on the head in addition to its inputs from second-order ocellar neurons. I found that the specificity of spike timing in DNI's response to fluctuating light stimuli was preserved when the neuron was excited either by directing puffs of air at the head or by depolarizing current injected into its cell body. Second, changes in temperature could alter the coding strategy, for example by significantly increasing spike frequency at temperatures a free-flying locust would encounter naturally. I found that increasing temperature does significantly increase the frequency response of DNI and its presynaptic second-order neurons. Although elevated temperatures increase the number of spikes during repeated stimuli, the precision in the timing of individual spikes also increases. At a temperature of 35ºC, DNI could continue to use timing to individual spikes during a wingbeat cycle to signal changes in horizon position.

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Xxxxxx **P 391**

I spy with which little eye? Range-finding in jumping spiders

Jumping spiders (Salticidae) are active hunters that rely primarily on vision to navigate their environment, locate and finally pounce on their prey. Measuring <10mm, salticids identify and stalk their prey based on information gathered by four pairs of specialised eyes, of which the two forward-facing pairs, the anterior-medial (AM) and anterior-lateral (AL) eyes, may be especially important for mediating depth perception. Depth judgements can be achieved through a variety of mechanisms, ranging from motion parallax to binocular overlap. The latter has typically been assumed to be used by salticids. We investigated if one of these pairs of eyes (and which) is sufficient and necessary for range-finding, or alternatively, if this judgment is mediated by both AM and AL eyes. We tested salticids whose eyes were selectively blinded using dental silicone in a purpose-built arena in which vergence cues were absent and which forced spiders to make an accurately gauged leap onto a platform. The distances and sizes of the platforms were altered such that they subtended the same visual angle, allowing us to test the mechanism used by salticids in making depth judgements, and also which eyes are used for this job.

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P 392

High acuity motion detection by the not so secondary eyes of jumping spiders

Jumping spiders are highly vision-dependent animals. Their modular visual system is both unique in design and outstanding in performance. This visual system consists of a pair of forward-facing 'principal' eyes with narrow fields of view, but exceptional spatial resolution, and two or three pairs of 'secondary' eyes with wide fields of view that function primarily as motion analyzers. Motion detected by the secondary eyes may elicit an orienting response, whereupon the object of interest is examined further using the high-acuity principal eyes. The antero-lateral (AL) eyes are particularly interesting, as they are the only forward facing pair of secondary eyes. We isolated the AL eyes by covering all other eyes with dental silicone. The spiders were then suspended facing computer screens, holding a polystyrene ball. We recorded optomotor responses of male and female spiders to computer-generated dot stimuli that varied in size and contrast, and moved at different speeds, to determine how stimulus parameters affect orientation behaviour. All three parameters had significant effects. Additionally, we looked for sex-based differences, and analysed the influence of different hunger states on orientation propensity. In general, female spiders responded more often than males, both when when sated and when hungry, but hunger lead to a similar percentile increase in orientations in both sexes. This might be an adaptation to different energy demands, a sex-based physiological difference in the visual systems, or related to the elaborate courtship displays of male jumping spiders. The AL eyes' role in the visual system appears to be more complex than previously assumed: When we provided tethered flies as prey, we found that visual information from the AL eyes alone was sufficient to elicit stalking behaviour, which underlines the relevance of the high spatial acuity of the AL eyes for overall visual processing.

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Slow facilitation of small target motion responses

Many insect species pursue small moving targets, e.g. the predatory dragonflies chase and capture small prey. To overcome the inherently limited resolution of the compound eye, many insects that pursue targets have developed acute zones. The optical specializations are accompanied with neural hardware giving selective responses to small targets. In the dragonfly lobula (3rd optic ganglion) small target motion detectors (STMD) respond to small moving targets (1-3º), with no response to larger bars or to widefield stimuli (1,2). STMDs are exquisitely sensitive neurons, detecting targets with an effective neural contrast as low as 2% (3). This performance is especially impressive considering the ability to respond to small targets against moving backgrounds, and the lack of sustained responses to clutter within moving natural scenes. What mechanisms provide STMDs with a high-enough gain to allow responses to low-contrast targets, while still avoiding breakthrough responses to target-like features in natural backgrounds? It is possible that a summation mechanism plays a role, enabling responses to build up as targets move continuously across the receptive field. To investigate the presence of facilitation mechanisms, we record intracellularly from the recently characterized Centrifugal STMD1 (CSTMD1) (2,4). Its large centrifugal axon allows for more stable recordings than usual for STMDs. We show that CSTMD1 has a slow facilitation mechanism where responses continue to grow for several hundred milliseconds as targets move across the receptive field. This gives a partial explanation for the ability of STMDs to respond to low contrast targets: only continuous motion of a target with the correct spatiotemporal profile allow responses to continue to build up to maximum firing frequencies. References: 1. O"Carroll. 1993. Nature 362, 541. 2. Geurten, et al. 2007. J Exp Biol 210, 3277. 3. Nordström, et al. 2006. PLoS Biol 4, 378. 4. Bolzon, et al. 2009. J Neurosci 29, 14143.

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P 394

The responses of target-selective descending neurons in the dragonfly to 3-dimensional object movements outdoors under blue sky

Dragonflies make their living by foraging on flying insects. Eight pairs of identified neurons are implicated in controlling the dragonfly's flight path as it intercepts its flying prey. These target-selective descending neurons (TSDNs) descend from the brain of the dragonfly to the thoracic ganglia. They show directionally selective responses to small objects moving relative to the dragonfly. Their receptive fields are located in the dorso-frontal quadrant of the visual field, the region that views prey during the foraging flight. When stimulated intracellularly with high-frequency, depolarizing current pulses, each of these neurons evokes small adjustments in wing position and attitude. To understand the behavior of the TSDNs under more natural environmental conditions, we studied their responses outdoors, under blue sky, to the movement of opaque white beads of three sizes (2, 4, and 8 mm) around the immobilized dragonfly. The bead movements were videotaped (100 frames/s) for 3-dimensional reconstruction of their paths. The extracellularly recorded TSDN spikes were sorted and correlated with bead positions and velocities. We recorded from dragonflies of two genera: Aeshna (which forages from continuous flight) and Pachydiplax (which takes off to forage from a perch). The outdoor recordings revealed several new properties of the neurons and their receptive fields, three of which are presented here. (1) Receptive fields are not identical between genera, a result that may be related to their markedly different foraging strategies. (2) Three-dimensional receptive field reconstruction showed that size selectivity varies with object distance. (3) The higher light levels and ambient temperatures outdoors resulted in TSDN spike rates of 900 Hz or greater, much higher than have ever been observed in laboratory experiments.

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3D reconstructions and flight statistics of dragonfly prey-capture trajectories

Dragonflies are among evolution's finest aerial hunters; intercepting prey in mid-air, they rarely miss their targets. Most previous studies of dragonfly prey capture have taken place outdoors and have been limited by environmental variability and availability of adult dragonflies in these situations. These single camera studies have been restricted to 2D analyses, and have missed long complex flights due to the narrow field-of-view (FOV) required by outdoor conditions. To enable more sophisticated studies of prey capture, we have constructed a fully indoor flight arena that can be used to study dragonfly behavior in a controlled environment. The flight arena is 5.5m x 4m x 4.5m in size, and is illumined to 10mW/cm2. The end result is a bright, windless, temperature and humidity controlled environment in which adult dragonflies can forage year round. Individual animals gained up to 100mg of weight per day, and lived as long as 3 weeks. We have focused our studies on libellulid dragonflies (L. Lydia, L. Luctuosa), because they will forage readily from a single perch positioned in the focal zone of a high speed camera array (2 cameras; 1000fps). We have analyzed over 150 prey capture trajectories of both the dragonfly and its prey (drosophila) in a 3D recording volume of ~1m3, with sub-millimeter tracking accuracy. Foraging success rates were ~80%, with a mean flight time of 361 ± 124ms – on the order of 15 wing strokes. Dragonfly flight accelerations were very high, over double those of its prey (dragonfly: mean acceleration 18 m/s2, max 75 m/s2 ; drosophila: mean acceleration 8 m/s2, max 42 m/s2). When the dragonfly failed to catch its prey, mean flight times increased by 20%, and mean accelerations increased by 10% (dragonfly) and 30% (drosophila). The data suggest the presence of a narrow "trigger zone" above and slightly forward of the dragonfly's head, which most prey pass through in the moments before a foraging flight begins.

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P 396

Attack of the Dragonfly: receptive fields and anatomy of the Target Selective Descending Neurons.

Dragonflies detect prey against a clear sky and then follow an interception trajectory to capture their target. These flights typically last 350ms from take-off to interception, and the accuracy afforded by the dragonfly retina is paramount for success. Despite this, little is known about the spatial and temporal response properties of the neurons underlying this behavior. We have begun to record intracellularly in libellulid dragonflies (L. lydia and L. luctuosa) from a class neurons that respond solely to small moving targets (Olberg, 1986). Using a custom-built 360Hz DMD projector, we have begun to make 0.25° resolution measurements of target-selective descending neuron (TSDN) spatiotemporal receptive fields, in an effort to develop quantitative circuit models of these cells. After mapping a TSDN receptive field, the cell is filled with a tracer dye (Lucifer yellow and/or neurobiotin) to confirm its identity, its three-dimensional structure, and its pre- and post-synaptic targets. Standard histological techniques do not adequately preserve the fragile structure of the dragonfly nerve cord. In libellulids, the diameter of TSDN axons ranges from 16-25um, while the cervical connective is only 230um wide - over 1/3 the volume of the cord is comprised of the cytosol within these axons. Consequently, it is necessary to embed the cord in resin to prevent rupture during semi-thin sectioning. The dehydration required for resin causes major deformation and shrinkage of the cervical connective in protocols in which osmium is omitted to preserve fluorophore signals. Whole-mounted cords are sufficiently transparent for 2-photon imaging, but the brain and ganglia are too thick to be optically sectioned in this manner. We will discuss the development of a tissue clearing protocol suitable for large insects, that preserves the anatomy of the ventral cord, keeps background fluorescence low, fluorophore signal high and antigenicity intact.

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P 397

In vivo Ca++ imaging responses to visual stimuli in columnar elements of the optic neuropils of an arthropod

Experiments with insects and crabs demonstrate their remarkable capacity to learn and memorize visual features. However, brain areas and neurons underlying these processes remain unknown. In the crab Chasmagnathus, a visual danger stimulus (VDS) elicits animal escape which declines after a few stimulus presentations giving rise to a short or long term memory depending on the training protocol experienced. In vivo intracellular recordings in the optic lobe of the crab show that this well studied learning and memory process can be extensively accounted for by plastic changes in lobula giants (LGs) movement detector neurons. However, a number of evidences suggest that the changes induced by massed training are presynaptic to the LG neurons. In arthropods, visual information is conveyed to the wide tangential neurons of the lobula by retinotopic narrow field columnar elements, which due to their small calibre are difficult to investigate with electrophysiological recordings. Thus, to investigate the possibility of plastic changes occurring in these columnar elements, here, we developed a preparation that allows assessing the response of these elements to visual stimuli using Ca++ imaging in the intact animal. First, as the retinal maps of crab optic neuropils were unknown, based on detailed tracing from retinal photoreceptors and neuronal projections along the optic neuropils we disclosed the crab retinal maps. Unexpectedly, we found that in the crab the chiasma between lamina and medulla reverses the order of horizontal retinotopic columns while the chiasma between medulla and lobula reverses the order of vertical columns. Second, to perform Ca++ imaging in the intact animal the crab was restrained and a small window was opened on the cuticle of the eyestalk. Trough this window, columnar neurons were stained with dextran Calcium Green, a Ca++ sensitive dye. We found consistent and clear responses from narrow field columnar elements from the lobula to different visual stimuli, including the VDS. The analysis of correspondence between the areas of columnar activation and the positions of stimulus presentation confirm the retinal maps derived from the anatomical studies. Ongoing studies are aimed at studying if these peripheral columnar elements are able to undergo plastic changes when confronted with repetitive presentation of a VDS.

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641 **P 398**

Visually mediated decisions of the escape direction in the crab Chasmagnathus

A critical feature of evasive behaviors is the ability to localize the threatening stimulus in space and perform consequently. When no shelter is available, crabs react to a sudden approaching object by running in the direction opposite to this looming stimulus. However, when an object is coming straight from above, the information about the proper side to run becomes less certain. Using this condition, we began to investigate what kind of contextual visual information may affect the decision for the direction of escape. The study is performed by using a running simulator device located inside an arrangement of 5 computer screens (1 above and 1 on each side of the simulator). This allows us to precisely record the course of the escape response elicited by a looming stimulus in the upper screen, and to investigate how it is affected by changes in the contextual visual environment. We found that when the luminance of the four screens surrounding the crab at the horizontal level where equal, 50 % of the animals ran to the left and the other 50 % ran to the right. Upon repeated stimulus presentations each individual tended to maintain the same escape direction, indicating the existence of individual directional preferences. On the other hand, when animals faced visual contextual asymmetries between the horizontal screens, like differences in luminance, polarization pattern or object form, the side preferences were clearly biased. Results show that when deciding the direction for escaping in the absence of a shelter, crabs take into account the predator's direction but also contextual visual information. By using in vivo intracellularly recording we have previously shown that lobula giant neurons play a key role in visually elicited escape behaviors. The present behavioural results give us now the possibility of exploring to what extent the decision for the escape direction takes place in these lobula giant neurons.

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Sequence and expression pattern of three opsins in the retina of an Atlantic sand fiddler crab, Uca pugilator

Fiddler crabs are intertidal brachyuran crabs that belong to the genus Uca. Approximately 97 different species have been identified, and several of these live sympatrically. Many have species-specific body color patterns that may act as signals for intra- and interspecific communication. To understand the behavioral and ecological role of this coloration we must know whether fiddler crabs have the physiological capacity to perceive color cues. Using a molecular approach, we identified the number of opsins expressed, and their expression patterns in the photoreceptor cells and ommatidia across the eye of the sand fiddler crab, Uca pugilator. We identified three different opsin-encoding genes (UpRh1, UpRh2 and UpRh3). UpRh1 and UpRh2 show an amino acid sequence similarity to other arthropod long- and medium-wavelength sensitive opsins, whereas UpRh3 is similar to other arthropod UV sensitive opsins. All three opsins are expressed in all ommatidia, and within each ommatidium an opsin-specific expression pattern is observed. UpRh3 opsin is present only in the R8th photoreceptor cell, whereas UpRh1 and UpRh2 are present in the R1-7 cells, with UpRh1 expression restricted to five cells and UpRh2 expression present in three cells. Thus there is one photoreceptor in every ommatidium expressing both UpRh1 and UpRh2. These results show that U. pugilator has the basic molecular machinery for color perception.

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