



## Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

### A10 – INVERTEBRATE MODELS OF BEHAVIOURAL AND CIRCUIT PLASTICITY

#### A10.1

09:00 Tuesday 30th June 2009

**Electrical and molecular changes contributing to associative memory in the pond snail *Lymnaea***

Paul R. Benjamin (University of Sussex)

The neural substrate for long-term memory (LTM) formation involves an ensemble of changes at the molecular, cellular and networks levels. How these changes are integrated to produce behavioural plasticity are largely unknown. By focussing on specific examples of these processes in the snail, *Lymnaea*, we have been able to make progress in understanding how changes at these different levels of organization contribute to memory. In the feeding system, classical reward conditioning has involved the use of tactile and chemical cues. One-trial classical conditioning is effective and produces LTM traces that last for up to 20 days. Examples of synaptic and non-synaptic and molecular changes induced by conditioning will be given. Non-synaptic changes include persistent changes in membrane potential that reduce the threshold for firing in intrinsic command-like neurons (tactile conditioning) and pre-synaptic facilitation of the CS pathway due to the depolarization of extrinsic modulatory interneurons (chemical conditioning). The biophysical processes underlying the extrinsic modulation of learning are beginning to be understood and involve changes in the strength of a persistent sodium current. Nitric oxide is required for LTM in chemical conditioning and levels of nNOS sense and antisense NOS RNAs are altered within the same critical 6 h following training suggesting that the requirement for NO in memory formation is accompanied by specific gene regulation. Understanding how these different electrical and molecular processes are integrated together is a major problem in memory research and is likely to require a computational analysis of the processes involved.

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#### A10.2

10:30 Tuesday 30th June 2009

**Optophysiological approaches to dissect neuronal circuits underlying learning and memory in *Drosophila***

Andre Fiala (Georg-August-University of Goettingen)

For many years *Drosophila* has been the geneticist's dream but the physiologist's nightmare, mainly due to the small size of central neurons that makes them almost inaccessible for extensive electrophysiological studies. However, novel genetic tools might help to combine the advantages of *Drosophila* – most notably the possibility to target defined neurons – with physiological techniques. We are using on the one hand DNA-encoded fluorescence sensors to monitor neuronal activity, on the other hand a light-sensitive ion channel to depolarize neurons by illumination.

Using these optical imaging and optical activation techniques we investigate how behaviour is influenced by modulatory neurons. In particular we follow up the hypothesis that modulatory systems might mediate the evaluation of external stimuli as indicative for positive or for negative outcomes, respectively.

Using optical calcium imaging of dopaminergic neurons innervating the mushroom body we show that these neurons are responsive to electric shocks, which are commonly used as punishing stimuli during olfactory associative training procedures. Light-activation of dopaminergic neurons in the larval *Drosophila* brain using channelrhodopsin-2 can substitute for the reinforcing effect of a punitive salt stimulus in an olfactory associative learning paradigm. Activating octopaminergic and/or tyraminerbic neurons substitutes for a rewarding stimulus in appetitive learning.

These experiments indicate that biogenic amines might act as positive or negative reinforcers in associative learning tasks in insects. Arguments for and against this model will be discussed.

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**A10.3****11:00 Tuesday 30th June 2009****Peptidergic neuromodulation in olfactory circuits of the *Drosophila* brain**

Dick R. Nässel (Stockholm University), Mikael A. Carlsson (Stockholm University), Asa M. Winther (Stockholm University)

Neuropeptides are likely to be important in neuronal modulation and plasticity. Most of the neuropeptide genes identified in *Drosophila* are expressed in the brain and many of the peptide products of these genes are produced by central interneurons. Although there is a large number of different neuropeptides in the *Drosophila* brain, relatively few peptides have been detected in each neuronal subsystem (neuropil region). As an example, the olfactory neurons of the antennal lobes seem to utilize peptide products of only three genes and the mushroom bodies that of one gene. Neuropeptide signaling in the olfactory system is tractable for functional analysis by means of targeted Gal4-UAS mediated interference, and we can benefit from great advances that have been made in understanding the processing of odor information. We approach the system both at the level of the first synapses in the antennal lobes and more centrally in the mushroom bodies. Two peptides, and their receptors, will be discussed here, short neuropeptide F (sNPF) and *Drosophila* tachykinin (DTK). A subset of the olfactory receptor neurons (ORNs) of antennae and maxillary palps expresses sNPF, and DTK signaling is seen in a feedback from local antennal lobe interneurons onto ORNs. The possible roles of these peptides in olfaction will be discussed. Furthermore, we have found that most of the intrinsic neurons of the mushroom bodies, the Kenyon cells, express sNPF and the functional consequences of these are under study.

Email Address for correspondence: [dnassel@zoologi.su.se](mailto:dnassel@zoologi.su.se)doi:[10.1016/j.cbpa.2009.04.294](https://doi.org/10.1016/j.cbpa.2009.04.294)**A10.4****11:30 Tuesday 30th June 2009****Glutamate uptake system in *Lymnaea stagnalis***

Etsuro Ito (Tokushima Bunri University), Dai Hatakeyama (Tokushima Bunri University), Koichi Mita (Tokushima Bunri University), Suguru Kobayashi (Tokushima Bunri University), László Hiripi (Balaton Limnological Research Institute), Károly Elekes (Balaton Limnological Research Institute)

A series of previous data derived by the aspects of glutamate and its receptors suggested that glutamate is used as a neurotransmitter for the generation of feeding rhythm in the pond snail *Lymnaea stagnalis*. The uptake mechanism of glutamate, however, is not yet known, and so in the present study we characterized glutamate transporters and examined the functions of these transporters in the feeding circuits of *Lymnaea* central nervous system (CNS). First, measuring the accumulation of <sup>3</sup>H-labeled glutamate showed the presence of glutamate transport systems with different types of glutamate transporters, excitatory amino acid transporter (EAAT) and vesicular glutamate transporter (VGLuT), in *Lymnaea* CNS. The highest accumulation rate was measured in the buccal ganglia, suggesting the involvement of glutamate transport systems in the feeding behavior. Second, we succeeded in cloning 2 types of glutamate transporter of *Lymnaea*, LymEAAT and LymVGLuT, and showed that the functional domains in both types of transporters are well conserved in comparison to these amino acid sequences with those of mammalian EAATs and VGLuTs. Third, in situ hybridization showed that the mRNAs of LymEAAT and LymVGLuT are transcribed in the major

feeding motoneurons in the buccal ganglia. Finally, the changes in firing patterns of the feeding motoneurons, which have glutamatergic inputs, by inhibition of LymEAAT were similar to those by stimulating with glutamate. These results showed that glutamate uptake systems are located in the feeding circuits and they are required for the generation of feeding rhythm of *Lymnaea*.

Email Address for correspondence: [eito@kph.bunri-u.ac.jp](mailto:eito@kph.bunri-u.ac.jp)doi:[10.1016/j.cbpa.2009.04.295](https://doi.org/10.1016/j.cbpa.2009.04.295)**A10.5****11:50 Tuesday 30th June 2009****Comparison of aversive and reward one-trial classical conditioning in the *Lymnaea* feeding system**

Ildiko Kemenes (University of Sussex), Michael O'Shea (University of Sussex), Paul R. Benjamin (University of Sussex)

We previously developed a one-trial reward classical conditioning paradigm using amyl-acetate as the conditioned stimulus (CS) and sucrose as the unconditioned stimulus (US), to study the electrical and molecular changes underlying long-term memory formation in the feeding system of the pond snail *Lymnaea*. Here, we compare the features of one-trial reward conditioning with an alternative aversive conditioning paradigm where quinine was used as the US while the CS was the same (amyl acetate) as in reward conditioning. Here, a single pairing of CS and US led to aversive conditioning. An electrophysiological correlate of both types of conditioning was measured in semi-intact preparations. The same CS caused excitation or inhibition depending on the type of the training. The source of the inhibition in case of aversive conditioning appears to be in non-feeding ganglia in the rest of the brain. Our results indicate that the same CS can trigger alternative responses depending on the type of training (US). These two types of responses involve different pathways within the CNS.

The sensitivity of the aversive and appetitive memory trace to NO, dopamine and octopamine blockers was also studied. We established that while NO and dopamine are important in the development of a long-term appetitive memory trace at an early stage after conditioning they do not play a role in aversive memory formation. Octopamine, on the other hand is not needed for appetitive memory formation, but seems to be the key transmitter needed shortly after aversive training.

Email Address for correspondence: [I.Kemenes@sussex.ac.uk](mailto:I.Kemenes@sussex.ac.uk)doi:[10.1016/j.cbpa.2009.04.296](https://doi.org/10.1016/j.cbpa.2009.04.296)**A10.6****13:30 Tuesday 30th June 2009****Genetic dissection of associative and non-associative learning in *C. elegans***

Catharine H. Rankin (University of British Columbia)

*Caenorhabditis elegans* are able to show a variety of forms of associative and non-associative learning as well as long-term memory for mechanosensory habituation. Our data suggests that there are multiple mechanisms mediating short-term habituation, and that there are several different forms of lasting memory. Protein synthesis dependent long-term memory for habituation in *C. elegans* is produced by spaced, but not massed training protocols. This long-term memory is mediated by CREB dependent down-regulation of a

Kainate/AMPA type glutamate receptor subunit, *glr-1*. Long-term habituation training is hypothesized to lead not to a change in the number of synapses, but to a decrease in the average size of synapses. A second, protein synthesis independent form of lasting memory can be seen 12 h after massed training. This memory is dependent on an increase in the release of an inhibitory neuropeptide from the sensory neurons. We have also shown associative learning in the form of context dependent habituation. In this procedure animals that are trained and tested in the same environment (chemosensory cue) show greater memory for training than worms trained and tested in different environments. We are using this paradigm to differentiate genes involved in associative and non-associative learning. We have found that a *C. elegans* homologue of AMPA/Kainate type glutamate receptors is critical for long-term memory for habituation, and that a homologue of NMDA-type glutamate receptors is critical for context conditioning. These data support the hypothesis that many mechanisms and characteristics of memory are highly conserved across evolution.

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#### A10.7

**14:30 Tuesday 30th June 2009**

##### **Synaptic organization and function in a single behaviorally relevant neuron**

Jann W. Gardner (University of Utah), Andres Villu Maricq (University of Utah), David M. Madsen (University of Utah), Frédéric Hörndli (University of Utah), Jerry E. Melleme (University of Utah), Craig S. Walker (University of Utah)

Many animals can associate temperature with favorable environmental resources and thermotaxis to the most favorable temperature. However, the neural mechanisms that regulate thermotaxis are not well understood. In the nematode *C. elegans*, a small neural circuit consisting of sensory neurons and interneurons detects temperature and directs movement. Our goal is to understand how this simple circuit functions at the level of individual synapses and receptors. A critical element in the thermotaxis circuit is the pair of RIA interneurons, which appear to have a major integrating function in determining the appropriate motor output. We have discovered that glutamatergic signaling mediated by the kainate class of ionotropic glutamate receptors (iGluRs) is required for thermotaxis. These kainate iGluRs are exclusively expressed in RIA, which receive inputs from the thermal sensing circuitry. Deletion mutations in kainate receptor subunits eliminate a subset of the glutamate-gated current in RIA and modify the thermotactic behavior. In contrast, no change in this behavior is observed in mutants that lack AMPA-class iGluRs. Thus, a single neuron differentially localizes classes of iGluRs to subserve information processing and control of behavior.

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#### A10.8

**15:40 Tuesday 30th June 2009**

##### **Hangovers, hairy dogs and worms: Modeling alcohol induced plasticity in *C. elegans***

Lindy Holden-Dye (University of Southampton), James Dillon (University of Southampton), Philippa Mitchell (University of South-

ampton), Yiannis Andrianakis (University of Southampton), Richard Mould (University of Southampton), Steven Glautier (University of Southampton), Christopher James (University of Southampton), Vincent O'Connor (University of Southampton)

The ability of neural systems to undergo homeostatic adaptation in response to chronic ethanol exposure is well-documented in both invertebrates and mammals. Unpicking the precise molecular and cellular determinants of the adaptive responses to ethanol is confounded by the interaction of ethanol with multiple neural signalling pathways. Here we define a paradigm in which the ability of the simple genetically tractable animal *C. elegans* to perform an integrative behavioural task, navigation towards food, provides a read-out of distinct ethanol-induced behavioural states. In particular we show that chronic exposure to, and subsequent withdrawal from, ethanol impairs navigational performance. Evidence that this is due to a direct neuroadaptive response to ethanol is provided by the observation that the behaviour in ethanol withdrawal is alleviated by a sub-intoxicating dose of ethanol. This 'withdrawal relief' effect substantiates the use of this behavioural paradigm to define neural substrates of ethanol-induced plasticity.

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#### A10.9

**16:20 Tuesday 30th June 2009**

##### **Cockroaches keep predators guessing by using preferred escape trajectories**

Paolo Domenici (CNR-AMCICM, Italy), David Booth (University of Sussex, UK), Jonathan M. Blagburn (University of Puerto Rico), Jonathan P. Bacon (University of Sussex, UK)

Anti-predator behaviour is vital for most animals, and calls for accurate timing and swift motion. Whereas fast reaction times and predictable, context-dependent, escape-initiation distances are common features of most escape systems, previous work has highlighted the need for unpredictability in escape directions, in order to prevent predators from learning a repeated, fixed pattern. Ultimate unpredictability would result from random escape trajectories. Although this strategy would deny any predictive power to the predator, it would also result in some escape trajectories towards the threat. Previous work has shown that escape trajectories are in fact generally directed away from the threat, although with a high variability. However, the rules governing this variability are largely unknown.

Here, we demonstrate that individual cockroaches (*Periplaneta americana*, a much-studied model prey species) keep each escape unpredictable by running along one of a set of preferred trajectories at fixed angles from the direction of the threatening stimulus. These results provide a new paradigm for understanding the behavioral strategies for escape responses, underscoring the need to revisit the neural mechanisms controlling escape directions in the cockroach and similar animal models, and the evolutionary forces driving unpredictable, or 'protean', anti-predator behaviour.

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**A10.10****16:40 Tuesday 30th June 2009****Environmental calcium concentration affects long-term memory formation in a freshwater gastropod**

Sarah Dalesman (University of Calgary), Ken D. Lukowiak (University of Calgary)

In the freshwater environment availability of dissolved calcium limits the distribution of many aquatic organisms. Data suggest this is due to the direct effect of calcium availability on growth rate and reproduction. One group of species in which this has been clearly demonstrated is the freshwater pulmonates. For calciphiles such as *Lymnaea stagnalis* environmental calcium concentration is considered to be the major abiotic limitation on their distribution, with a lower limit of 20 mg/l being cited in the U.K. Here we show for the first time that *L. stagnalis* maintained for even short periods in low calcium concentrations demonstrate a reduced capability of forming memory following associative learning (operant conditioning of aerial respiration). Snails maintained at high  $[Ca^{2+}]$  80 mg/l demonstrate both intermediate (ITM; 2–3 h; dependent on de novo protein synthesis) and long term (LTM; 24 h; dependent on altered gene activity and new protein synthesis) memory. However, following short term exposure (1 h) to low  $[Ca^{2+}]$  20 mg/l they are only able to form ITM. Conversely, snails maintained at  $[Ca^{2+}]$  20 mg/l rapidly regain the ability to form LTM following a short period of exposure to high  $[Ca^{2+}]$  80 mg/l. Low environmental  $[Ca^{2+}]$  may be preventing the required transcription necessary for LTM formation but does not appear to inhibit the de novo protein synthesis necessary for ITM formation. Thus, despite this species surviving at low  $[Ca^{2+}]$  20 mg/l they exhibit less adaptability as indicated by a decreased capability of forming memory following the acquisition of a new behaviour.

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**A10.11****09:00 Wednesday 1st July 2009****Watching the brain of the honeybee as it learns and remembers**

Randolf Menzel (Free University Berlin), Paul Szyszka (University Konstanz)

Honeybees discriminate a large range of odors, and learn odors as signals for food. We have used optical imaging, extra- and intracellular recording techniques to examine the neural substrates of odor coding and memory formation in the primary and secondary neuropil of the honeybee olfactory system, the antennal lobe and the mushroom bodies. Odors elicit combinatorial patterns of activity in the glomeruli of the al. These patterns can be visualized using calcium-sensitive dyes backfilled via particular nerve bundles. The responses of the postsynaptic elements within the glomeruli allow the reconstruction of network properties of the al. Associative plasticity of the network in the antennal lobe has been found both by ca imaging and extracellular multielectrode recordings. Imaging of the input region of the mushroom bodies (the lip region) reveals that odors are represented in specific patterns of microglomerular activation. These patterns are partially overlapping and highly consistent. A comparison of the dynamics of the odor responses shows that kenyon cells code odors in a sparse way both in the time domain and on the population level. Odor learning leads to a change of the synaptic transmission at the input synapses to kenyon cells for the learned odors. Extracellular recordings from mushroom body

extrinsic neurons indicate that the mushroom body recodes odors as the consequence of learning. A model will be presented that captures the results of our electro- and optophysiological recordings, and assigns particular functions to the mushroom body in the bee brain.

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**A10.12****10:30 Wednesday 1st July 2009****Circadian modulation and signaling pathways in associative learning: Lessons from *Aplysia***

Lisa C. Lyons (Florida State University), Maximilian Michel (Florida State University), Charity L. Green (Florida State University), Brianna Millsaps (Florida State University)

Identification of the processes through which memory may be modulated is a fundamental component in unraveling the mechanisms through which learning and memory occur. The marine mollusk *Aplysia californica* is a superb model for studies of learning, with extensive research by many scientists contributing to our understanding of the molecular and cellular mechanisms involved in memory formation. We have used *Aplysia* to investigate how the endogenous circadian clock modulates learning and the formation of memory *in vivo*. Previously, we found that the circadian clock strongly modulates long-term, but not short-term, memory formation for non-associative sensitization and the associative paradigm, learning that food is inedible (LFI; Fernandez et al., 2003; Lyons et al., 2005). Recently, we found that the circadian clock also modulates intermediate-term sensitization (Lyons et al., 2008). These behavioral studies have served to define the parameters of circadian modulation and underscored its importance. However, little information exists regarding the pathways through which the circadian clock impacts memory or the molecular mechanisms underlying LFI memory formation in *Aplysia*. We found that the MAPK, PKA, PKC and PKG signaling pathways are necessary for intermediate and long-term LFI memory. However, only MAPK appears necessary for short-term memory. Currently, we are investigating whether the circadian clock regulates the activation of these signaling pathways during learning and memory formation. We predict that robust circadian modulation involves multiple steps, particularly highly conserved signaling pathways, during the induction and formation of memory. Research is supported by NIMH Grant MH081012 to L.C.L.

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**A10.13****11:00 Wednesday 1st July 2009****Homeostasis versus neuronal variability: Models and experiments in crustaceans**

Thomas Nowotny (University of Sussex), Attila Szucs (UCSD), Rafael Levi (UCI), Allen I. Selverston (UCSD)

Invertebrate models, in particular the crustacean stomatogastric CPGs, have been excellent tools for studying the generation and modulation of rhythmic behaviors. In these invertebrate models, neurons are identifiable by their electrical activity and comparison to simultaneously recorded activity in identified nerves. The identifiable

cell has been a powerful concept enabling researchers to map cellular and network properties through repeated experimentation. Recently this approach has led to an interesting controversy on the consistency of cellular properties in identified neurons. Golowasch et al. (J. Neurophysiol. 87, 1129–1131 (2002)) report up to 3 fold variations in the maximal conductances of ionic currents in identified neurons in the crab STG across preparations. In a modeling study Prinz et al. (Nature Neurosci. 7(12):1345–1352 (2004)) furthermore demonstrated that multifold variations in parameters appeared to be consistent with a given set of output properties. I will present the evidence, some additional data from our own work (Nowotny et al. Neural Comput. 19:1985–2003 (2007)) and discuss the implications for our understanding of how systems are regulated to function properly. I will briefly discuss how these findings fit into the overall picture of regulatory mechanisms, the action of neuromodulators, feedback and descending control mechanisms and the time constants of ionic channels.

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#### A10.14

11:30 Wednesday 1st July 2009

##### The effect of opiate and opiate antagonists on the nocifensive response of nematodes to noxious thermal stimuli

Fernando E. Nieto-Fernandez (SUNY College at Old Westbury), Stephen C. Pryor (SUNY College at Old Westbury), Sybil Andrieux (SUNY College at Old Westbury), Ciara Bagnall (SUNY College at Old Westbury), Suhail Idrees (SUNY College at Old Westbury)

The antinociceptive effect of opiates in invertebrates is well documented in the literature. This is also true for parasitic and free living nematodes. The latency response of *Ascaris suum* to an aversive heat stimulus increased significantly when exposed to morphine, 4.93 to 12.96 s, and morphine 6 glucuronide, 5.17 to 9.28 s (10–20 nmol/mg). This effect was reversed by naloxone and CTOP for morphine and morphine 6-glucuronide respectively. In the case of *Caenorhabditis elegans*, morphine and the opioids endomorphin 1 and 2 also cause an antinociceptive effect on the thermal avoidance response (Tav). Adult wild type *C. elegans* N2 were collected from NGM plates using M9 buffer and exposed to morphine and endomorphine 1 and 2 in concentrations from  $10^{-4}$  to  $10^{-6}$  M (0.25 to 25 nmol/mg) for 30 min. The Tav behavior was then scored. In addition the effects of the opioid receptor antagonists Naloxone and CTOP were tested in combination with the drugs. Our results show that 46.56% of the morphine exposed worms exhibited a class I response versus 76.46% of the control group ( $P < 0.05$ ). Endomorphin 1 and 2 also caused a statistically significant reduction in class I responses, 35.99% and 39.16% respectively. Naloxone and CTOP blocked the analgesic effect of morphine, and endomorphine 1 and 2. These data appears to support the presence of a candidate mu opioid receptor gene in nematodes.

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#### A10.15

11:50 Wednesday 1st July 2009

##### Learning, aging and division of labour in honey bees

Ricarda Scheiner (Technische Universität Berlin)

Aging is accompanied by a decline of cognitive functions throughout the animal kingdom. But only few animal models have been studied for their biology of aging. Honey bees are emerging as a new model system for the study of aging and age-related learning deficits.

In honey bees, age is tightly linked to division of labour. Young bees work in the centre of the hive as nurses and take care of the brood and the queen. Old bees forage outside the hive for pollen and nectar.

We analysed how associative learning performance changes with age and social role in honey bees. In the first half of a honey bee's life, learning performance increases with age. In the second half, learning performance depends on social role rather than on age. Foragers show a decline in learning performance with increasing foraging duration. Nurse bees, in contrast, do not show any learning deficits even in high age. When foragers are artificially induced to revert to nursing tasks, their learning performance improves again.

These data imply a complex relationship between learning, aging and division of labour in honey bees. The improvement of learning performance in foragers which were reverted to an earlier behavioural state suggests a unique plasticity of aging and brain functioning in honey bees.

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#### A10.16

13:30 Wednesday 1st July 2009

##### Olfactory information processing, odor-modulated behavior, and their plasticity in the moth *Manduca sexta*

John G. Hildebrand (University of Arizona), Joshua P. Martin (University of Arizona), Carolina E. Reisenman (University of Arizona), Hong Lei, Jeffrey A. Riffell (University of Arizona)

We investigate odor-modulated behavior and neural processing of odor mixtures in *Manduca sexta*. Our goals are to understand: (a) the neurobiological mechanisms through which information about olfactory stimuli is encoded, processed, and integrated with inputs of other modalities in the moth's brain; (b) how the innate or learned behavioral significance of an odor is encoded in the brain; and (c) how this odor information ultimately initiates and controls characteristic behavioral responses. Insights from the sex-pheromonal communication system have led to recent analysis of odor-dependent moth–hostplant interactions. A multidisciplinary approach combining chemical characterization of natural volatiles, behavioral experiments in a laboratory wind tunnel, and electrophysiology has enabled us to determine how mixtures of volatiles, at natural concentrations, control flight behavior and are encoded in the antennal lobe of the brain. Mounting evidence points to coherent firing of output neurons of glomeruli as a mechanism for neural coding of the context or significance of an odor. Gas chromatography coupled with multi-channel CNS recording has enabled identification, in complex floral mixtures, of key odorants to which antennal-lobe neurons are particularly responsive. Mixtures containing only a few of those floral odorants are as effective as the complete, natural floral blend in modulating flight. Finally, when a moth learns to associate an arbitrary odor with a food reward, the ensemble of antennal-lobe neurons that gives phase-locked, consistent responses to that odor changes stably, and the newly “meaningful” odor evokes source-directed flight behavior. [Supported by NIH grant DC002751].

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#### A10.17

14:30 Wednesday 1st July 2009

### Dynamic molecular networks of memory formation in the honeybee

Uli Müller (Saarland University)

Memory formation is a dynamic and multiphasic process, highly conserved at the molecular level. In species as diverse as insects, molluscs and mammals parameters like number and temporal relation between training trials are critical for the induction of distinct memory phases. Associative appetitive learning in the honeybee provides the unique opportunity to identify and characterize the temporal and spatial features of the signalling network underlying the distinct memory phases in vivo. Our analysis revealed a network of parallel-organized molecular processes located in the antennal lobes and the mushroom bodies that account for distinct aspects of memory formation. Depending on the training procedure particular elements of this network are activated and thus contribute to induction and maintenance of the distinct memory phases. Interference with each of these distinct processes directly affects specific features of memory formation. Thus, the molecular network triggered by associative learning provides an ideal target to modulate and orchestrate different aspects of learning and memory formation. We are presently addressing the question of how intrinsic and extrinsic factors like stress, satiation, etc. interact with the molecular network underlying memory formation. Our findings suggest that the modulation of the learning machinery by intrinsic and extrinsic factors is due to the interaction of well-known evolutionary conserved molecular networks.

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#### A10.18

15:40 Wednesday 1st July 2009

### The phenotypic plasticity of swarm formation in the Desert Locust: Mechanisms and consequences

Swidbert R. Ott (University of Cambridge), Heleen Verlinden (University of Leuven), Stephen M. Rogers (University of Cambridge)

Animals can respond adaptively to changes in their environment by changing between alternative behavioural phenotypes. We seek to elucidate the underlying mechanisms of neural plasticity by combining analyses at the molecular, cellular, network and whole animal level in the Desert Locusts (*Schistocerca gregaria*). Locusts show a dramatic example of phenotypic plasticity, transforming between a cryptic solitary phase that shuns other locusts and a swarming gregarious phase depending on population density. Behaviour is key to both establishing and maintaining either of the two phases: a few hours of forced crowding with other locusts triggers a transition from strong mutual aversion to aggregation and greater activity. Subsequent changes in physiology and morphology follow from positive feedback. We have recently identified the neurochemical mechanism that underlies the initial transformation to gregarious behaviour. Defined sensory stimuli cause a transient increase in serotonin in the

thoracic ganglia that is both necessary and sufficient for behavioral gregarization to occur. Comparison of serotonin immunofluorescence in the ganglia of locusts subjected to different gregarizing regimes revealed individual neuronal somata that show long-term differences between phases, and others that up-regulate 5HT within 1 h in response to gregarizing stimuli. Pharmacological intervention, analysis of gene expression and dsRNA interference suggest a critical role for PKA in the acquisition and maintenance of the gregarious state. Inhibition of PKA, but not PKG, retards the transition to gregariousness in response to crowding, whereas dsRNA knock-down of the regulatory subunit PKA-R1 accelerates the process.

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#### A10.19

16:20 Wednesday 1st July 2009

### The role of PDZ scaffold CASK and CaMKII signalling in synaptic plasticity and learning of *Drosophila*

James J. Hodge (Bristol University)

CaMKII is an abundant kinase in the brain, constituting 1–2% of its total protein and is the main protein in the hippocampal post-synaptic density. Once activated by increased  $Ca^{2+}$ , CaMKII is able to cause a switch in its own activity (called T287 autophosphorylation), which allows it to have increased enzymatic activity independent of elevated  $Ca^{2+}$ . This special ability of CaMKII has been called the “molecular memory switch” and is required for LTP and learning. In addition CaMKII can mediate other changes that occur during synaptic plasticity and memory formation. These CaMKII mediated changes in synaptic plasticity are required for learning and memory in most animals including *Drosophila* (Griffith, J Neuroscience 2004).

Recently it has been shown that when  $Ca^{2+}$  is low (i.e. at quiescent synapses) CaMKII can regulate its own activity by a second mechanism (called T306 autophosphorylation) and this tends to inactivate CaMKII even if postsynaptic  $Ca^{2+}$  levels rise. The PDZ adaptor protein, CASK interacts with CaMKII at fly synapses and increases the ability of CaMKII to undergo T306 autophosphorylation and by doing so interferes with the ability of CaMKII to undergo T287 autophosphorylation (Lu et al., Neuron 2003; Hodge et al., Neuron 2006). This interaction would be predicted to interfere with LTP-like events and learning. We are using the genetic tractability of *Drosophila* to further determine the role of CASK and its regulation of CaMKII activity in synaptic plasticity underlying learning.

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#### A10.20

16:40 Wednesday 1st July 2009

### Multiple identified peptidergic interneurons express a novel pigment-dispersing hormone in the *Daphnia* brain and visual ganglia, some showing evidence for clock-neuron functions

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Postgenomic, precursor, expressed sequence tag, and mass spectrometric peptidomic analyses allowed us to identify a single gene leading to a novel 18mer-isoform of a bety-type pigment-dispersing hormone (PDH) that is identical in two waterflea species, *Daphnia magna* and *Daphnia pulex*. PDH is restricted to interneurons, ten types in the brain and two types in the visual ganglia, and does not occur in neurosecretory cells connected to neurohaemal areas. The neurons individually identified by immunohistochemistry (IHC) were virtually identical in terms of their positions and projection patterns in both species. Whereas the brain neurons are found associated with almost all major neuropils incl. the central body, the visual ganglia neurons adjacent to and innervating the medulla closely resemble the insect so-called pigment-dispersing factor medulla lateral neurons based upon positional and projection criteria. Since the latter neurons are established members of insect circadian clock systems, we analysed these neurons for IHC-detectable circadian changes. Preliminary results under 12:12 h light dark cycles showed significant circadian changes in numbers and staining intensities of the *Daphnia* medulla PDH neurons. These PDH neurons comprise a simple system currently studied in more depth in behavioural contexts. The discovered homologies to PDH-systems in decapod crustaceans and the well known clock system in several insects suggest evolutionary conservation of an ancient peptidergic interneuronal system in arthropods.

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#### A10.21

##### Poster Session – Tuesday 30th June 2009 Dissecting the resolution of a fruit fly retina

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The *Drosophila* visual system copes with the strains imposed by a small body size and successfully guides complex behaviors. To understand a visual system, knowing its acuity is paramount. The acceptance angle  $\Delta\rho$ , is the parameter used to describe spatial performance. Stavenga (2003) calculated  $\Delta\rho = 4.2^\circ$ . However, through *in-vivo* recordings of intracellular photoreceptor responses, we have measured  $\Delta\rho = 8.6 \pm 3$  (Mean  $\pm$  S.D.;  $n = 13$ ) in dark adapted flies. Such a disparity could be explained if the feedback synaptic connections in the fly eye (Meinertzhagen and Sorra, 2001) change the spatial properties of photoreceptor cells. To investigate this possibility, we aimed to “disconnect” the visual system from the input of photoreceptors R1–R6 exclusively at high temperatures and in a reversible manner by overexpressing *shibire*<sup>ts1</sup> (*shi*<sup>ts1</sup>) in photoreceptors. *shi*<sup>ts1</sup> causes synaptic vesicle depletion above 31 °C, but leaves neural communication intact at temperatures below 19 °C (Kitamoto, 2001).

Our results show that at the reported permissive temperature (19 °C), overexpressing *shi*<sup>ts1</sup> causes decelerated phototransduction and neurotransmission. Additionally, the photoreceptor cells appear enlarged and contain multi-vesicular bodies. Furthermore, the terminals of photoreceptors overexpressing *shi*<sup>ts1</sup> have a low vesicle count, apoptotic mitochondria and coated microtubules. Gold immuno-labeling confirmed that *shibire* protein coated the microtubules. Shpetner and Vallee (1989) reported the dynamin-microtubule interaction *in-vitro*, but to our knowledge this is the first study reporting such interaction *in-vivo*.

Kitamoto (2001). *J. Neurobiol.* 47:81-92. Meinertzhagen and Sorra (2001). *Prog. Brain Res.* 131: 53-69. Shpetner and Vallee (1989). *Cell* 59: 421-32. Stavenga (2003). *J. Comp. Physiol.* 189: 7245-7256.

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#### A10.22

##### Poster Session – Tuesday 30th June 2009

##### Function of the Shaw potassium channel within the *Drosophila* circadian clock

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In addition to the molecular feedback loops, electrical activity is important for the function of networks of clock neurons in generating rhythmic behavior. In order to determine the cellular mechanisms that regulate resting membrane potential (RMP) in the native clock of *Drosophila* we modulated the function of Shaw, a widely expressed neuronal potassium (K<sup>+</sup>) channel known to regulate RMP in *Drosophila* central neurons.

We show that Shaw is endogenously expressed in clock neurons. Differential use of clock gene promoters was employed to express a range of transgenes that either increase or decrease Shaw function in different clusters of clock neurons. Under LD conditions, increasing Shaw levels in all clock neurons, or in subsets of clock neurons (LNd and DN or DN alone) increases locomotor activity at night. In free-running conditions these manipulations result in arrhythmic locomotor activity without disruption of the molecular clock. Reducing Shaw in the DN alone caused a dramatic lengthening of the behavioral period. Changing Shaw levels in all clock neurons also disrupts the rhythmic accumulation and levels of Pigment Dispersing Factor (PDF) in the dorsal projections of LNV neurons. However, changing Shaw levels solely in LNV neurons had little effect on locomotor activity or rhythmic accumulation of PDF.

Based on our results it is likely that Shaw modulates pacemaker and output neuronal electrical activity that controls circadian locomotor behavior by affecting rhythmic release of PDF. The results support an important role of the DN clock neurons in Shaw-mediated control of circadian behavior.

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#### A10.23

##### Poster Session – Tuesday 30th June 2009

##### Transcriptional control of behavior: Engrailed knockout with RNAi changes cockroach escape trajectories

David Booth (University of Sussex), Bruno Marie (University of Puerto Rico), Paolo Domenici (CNR-AMC), Jonathan M. Blagburn (University of Puerto Rico), Jonathan P. Bacon (University of Sussex)

The cerci of the cockroach are covered with identified sensory hairs, which detect air movements. The sensory neurons which innervate these hairs synapse with giant interneurons (GIs) in the terminal ganglion which in turn synapse with interneurons and leg motoneurons in thoracic ganglia. This neural circuit mediates the animal's escape behavior. The transcription factor Engrailed (En) is expressed only in the medially born sensory neurons, which suggested it could work as a positional determinant of sensory

neuron identity. Previously, we used RNA interference (RNAi) to abolish En expression, and found that the axonal arborization and synaptic outputs of an identified En-positive sensory neuron changed so that it came to resemble a nearby En-negative cell, which was itself unaffected. We thus demonstrated directly that En controls synaptic choice, as well as axon projections.

Is escape behavior affected as a result of this mis-wiring? Adult cockroaches keep each escape unpredictable by running along one of a set of preferred escape trajectories (ETs) at fixed angles from the direction of the threatening stimulus. The probability of selecting a particular ET is influenced by wind direction. Early instar juvenile cockroaches use the same ETs as the adults and are amenable to RNAi. En knockout significantly perturbs the animals' perception of posterior wind, altering the choice of ETs to one more appropriate for anterior wind. This is the first time that it has been shown that knockout of a transcription factor controlling synaptic connectivity can alter the perception of a directional stimulus.

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#### A10.24

Poster Session – Tuesday 30th June 2009

##### Costs and benefits of predator induced behaviour in larvae of the urban mosquito (*Aedes notoscriptus*)

Vincent O. Van Uitregt (The University of Queensland), Robbie S. Wilson (The University of Queensland), Tim Hurst (Queensland Institute of Medical Research)

Prey often exhibit behavioural and morphological responses that convey greater survival in the presence of predators. The evolution and maintenance of such responses requires a functional trade-off between alternate phenotypes. That is, predator-adapted phenotypes must be beneficial in the presence of predators but costly in their absence. While the cost/benefit trade-off of prey responses seem intuitive, they are often difficult to demonstrate empirically. In this study, we examine the costs and benefits of the behavioural response of larval mosquitoes *Aedes notoscriptus* to fish predators. Larval *A. notoscriptus* reduce activity in the presence of predator chemical cues from firetail gudgeon, *Hypseliotris galii*. We will test the adaptive benefits of the behavioural response by entering predator exposed and naïve larvae into predation trials with *H. galii*. Fitness costs will be measured by comparing longevity and lifetime fecundity of predator exposed to predator naïve females. We predict that larvae exposed to predator chemical cues throughout development will avoid detection from *H. galii* for longer, but suffer a shorter adult life span and/or reduced lifetime fecundity. We will discuss the findings of these experiments and the potential use of aqueous predator chemical cues as control agents of pest mosquitoes.

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#### A10.25

Poster Session – Tuesday 30th June 2009

##### 3-Dimensional organisation of the antennal lobe of the parasitoid *Cotesia plutella*: A confocal microscopic study

Helga Groll (University of Southampton), Guy M. Poppy (University of Southampton), Phil L. Newland (University of Southampton), Ana Carolina Roselino (Université de Toulouse), Martin Giurfa (Université de Toulouse)

Parasitoid wasps rely on the detection of plant volatiles, odour compounds emitted by a plant after herbivore attack, to track down their host for successful reproduction. To determine how olfactory information is processed in their brain, it is necessary to describe the underlying anatomical brain structures. This study focuses on the antennal lobe of *Cotesia plutellae* and its glomerular organisation. Brain structures were stained with RH795 and observed by confocal microscopy. In addition, a 3D reconstruction of the antennal lobes was created using AMIRA software.

The average head size of *C. plutellae* was  $645 \pm 17 \mu\text{m} \times 446 \pm 14 \mu\text{m}$  ( $n = 16$ ), with two antennal lobes (AL) each measuring  $185 \pm 3 \mu\text{m} \times 139 \pm 3 \mu\text{m} \times 119 \pm 3 \mu\text{m}$  ( $L \times W \times D$ ) ( $n = 73$ ). The average volume of the AL, including measurements of females and males as well as both sides valued  $311.104 \mu\text{m}^3$ . There was no significant difference between length, width or depth neither of the antennal lobe nor between left and right size and gender.

The glomeruli were located in the outer part of the antennal lobe, around a central area devoid of any apparent glomeruli in the ventral sections. A mean number of  $54 \pm 7$  ( $n = 4$ ) was counted. Glomerular size was relatively constant throughout the different individuals, with an average diameter of  $22 \times 22 \mu\text{m} \pm 0.5$  ( $n = 67$ ). In addition to ordinary glomeruli, males had 3–4 enlarged ones ( $52 \pm 3 \times 31 \mu\text{m} \pm 2$ ) near the entrance of the antennal nerve, which leads to the speculation of a macroglomerular complex responsible for pheromone detection.

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#### A10.26

Poster Session – Tuesday 30th June 2009

##### Exploring visual motion circuitry in *Drosophila* with ultraviolet light

Trevor J. Wardill (University of Sheffield), Mikko I. Juusola (University of Sheffield)

Compared to higher organisms, the relatively simple and genetically malleable *Drosophila* can help us to make sense of motion coding strategies and visually driven behaviour. We wish to study how visual motion-sensitive information is routed to and processed in lobula plate tangential neurons (LPTNs) which express calcium sensitive dyes. For this purpose we have built a 2-photon imaging setup to record LPTN  $\text{Ca}^{2+}$  responses to moving patterns. Given that photoreceptor cells are likely to be excited by the  $\text{Ca}^{2+}$  fluorescence and by the high power infrared laser, we have created flies which have R1–R6 photoreceptors that are solely ultraviolet (UV) sensitive. Through epifluorescence imaging, we obtained preliminary  $\text{Ca}^{2+}$  changes in LPTNs in response to moving UV patterns *in vivo*.

To determine which neurons are involved in motion processing, we now wish dissect the bottom-up and top-down circuitry to LPTNs. Using a flight simulator, we have shown that the responses of UV-flies to UV stimuli correspond with wild-type responses to visible stimuli. Next,  $\text{Ca}^{2+}$  signals in UV sensitive LPTNs will be recorded and compared to other transgenic flies. We aim to change the signaling in LPTNs, to understand which synaptic inputs control LPTN activity, by ablating or by transgenic expression of light gated channels. Currently, we are working on expressing the light gated channel, Channel Rhodopsin2 (ChR2), to excite neurons. We are yet to see if ChR2 can influence UV-LPTNs. The results will be utilized to build realistic mathematical models of motion detection.

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