International Symposium in Honeybee Neuroscience
Berlin, June 10 - 13, 2010

Honeybee Neuroscience – a New, Old Model System, Bridging Genomics, Physiology and Behavior. Where to in the Next 50 Years?

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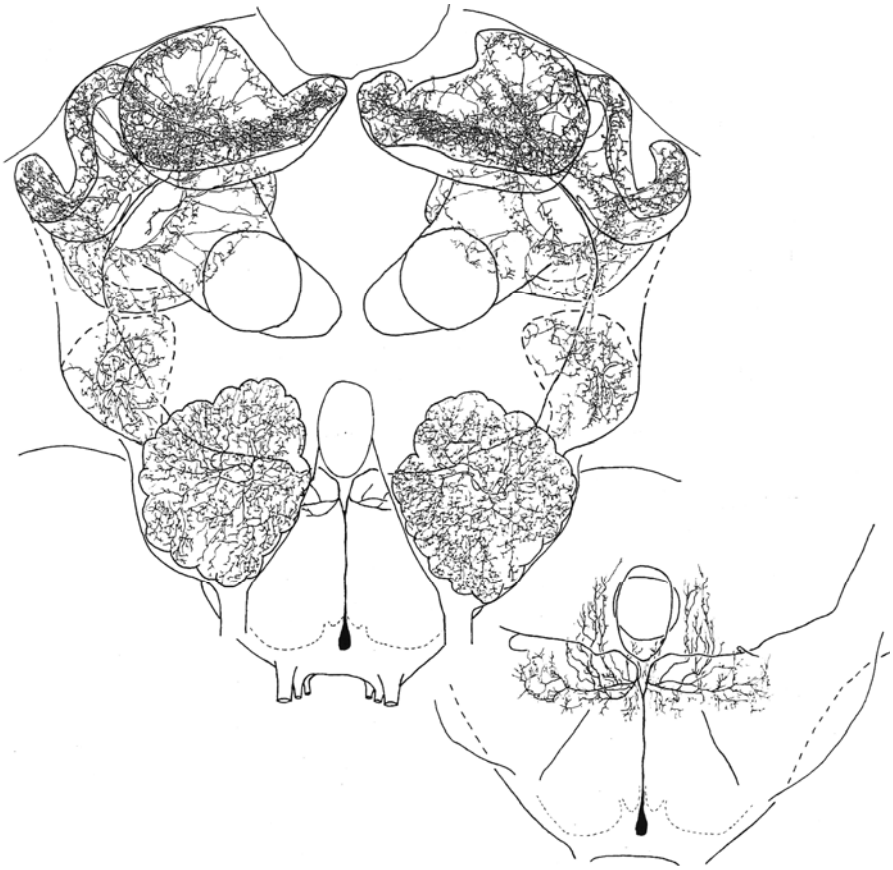

Honeybee Neurobiology and Behavior

- a Tribute for Randolf Menzel

Eds.: Dorothea Eisenhardt, C Giovanni Galizia, Martin Giurfa

Multi-authored book to be compiled from the symposium:
Honeybee Neuroscience, June 2010, Berlin.

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(Image of the VUMmx neuron from Martin Hammer, 1998)

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"Honeybee Neuroscience – a New, Old Model System, Bridging Genomics, Physiology and Behavior. Where to in the Next 50 Years?"

Zuse Institute Berlin (ZIB)

Thursday, 6/10/2010

16:00-17:00 *Registration and hanging of posters*

17:00-17:30 *Introductory remarks*
"What do learning and motor control have in common"
(Jochen Pflüger, Berlin, Germany)

17:30-19:00 *Plenary lecture*
Visual cognition in honeybees: from elemental stimulus learning and discrimination to non-elemental categorization and rule extraction
(Martin Giurfa, Toulouse, France)

19:00-19:30 *Meeting of the authors of the forthcoming book (Springer Publisher):*
"Honeybee neurobiology and behavior – a tribute for Randolf Menzel"

20:00 *Dinner and reception (at Seminaris hotel)*

Friday, 6/11/2010

08:30-08:45 *Opening remarks*
(Dorothea Eisenhardt, Berlin, Germany)

08:45-11:15 *Session 1: Sensory systems*
(Chair: Giovanni Galizia, Konstanz, Germany)

08:45-09:15 The auditory system of the honeybee
(Hiroyuki Ai, Fukuoka, Japan)

09:15-09:45 Chromatic and achromatic vision of bees in relation to flower patterns
(Misha Vorobyev, Auckland, New Zealand)

09:45-10:15 Psychophysics of honeybee colour processing in complex environments
(Adrian Dyer, Monash, Australia)

10:15-10:45 *Break and poster session*

10:45-11:15 Olfaction in the honeybee: multiple odour representations in the honeybee brain
(Jean-Christophe Sandoz, Toulouse, France)

11:15-15:05 *Session 2: Genetics and molecular biology*
(Chair: Jean-Christophe Sandoz, Toulouse, France)

11:15-11:45 Molecular dissection of the honeybee brain: an approach to solving the mystery of 'dance communication' and the sociality of the honeybee
(Takeo Kubo, Tokyo, Japan)

11:45-12:15 Elucidating the path from genotype to behaviour: is epigenomics a way forward for the honey bee neuroscience?
(Ryszard Maleszka, Canberra, Australia)

12:15-14:15 *Lunch (at FU Mensa)*

14:15-14:45 Molecular insights into honeybee brain plasticity
(Judith Reinhard and Charles Claudianos, Queensland, Australia)

14:45-15:05 Glutamate neurotransmission in the honeybee central nervous system
(Gerard Leboulle, Berlin, Germany)

15:05-16:35 *Session 3: Social organization within the hive*
(Chair: Judith Reinhard, Queensland, Australia)

15:05-15:35 The genetic and developmental evolution of social organization
(Rob Page, Tempe, USA)

15:35-16:05 Molecular genetic regulation of division of labor
(Gro Amdam, Tempe, USA)

16:05-16:35 The social regulation of task-related plasticity in circadian rhythms in honeybees
(Guy Bloch, Jerusalem, Israel)

16:35-17:05 *Break and poster session*

17:05-18:05 *Session 4: Communication within the hive*
(Chair: Gro Amdam, Tempe, USA)

17:05-17:35 Foraging honeybees: how foragers determine and transmit information about feeding site locations
(Harald Esch , Notre Dame, USA)

17:35-18:05 Olfactory information transfer during recruitment in honeybees
(Walter Farina, Buenos Aires, Argentina)

19:30 *Dinner (at Museum Dahlem)*

Saturday, 6/12/2010

08:30-10:30 *Session 5: Learning and memory*
(Chair: Guy Bloch, Jerusalem, Israel)

08:30-09:00 Universal laws of behavior tested in the honeybee
(Ken Cheng, Sydney, Australia)

09:00-09:20 Tactile learning in the honeybee
(Joachim Erber, Berlin, Germany)

09:20-09:40 Formation of contrasting memories in honeybees
(Dorothea Eisenhardt, Berlin, Germany)

09:40-10:00 Molecular biology of learning and memory: from memory phases to signaling cascades
(Uli Müller, Saarbrücken, Germany)

10:00-10:30 Distributed plasticity in the honeybee brain
(Brian Smith, Tempe, USA)

10:30-10:50 Break

10:50-14:40 Session 6: Brain anatomy and physiology
(Chair: Dorothea Eisenhardt, Berlin, Germany)

10:50-11:10 The honeybee standard brain
(Jürgen Rybak, Berlin, Germany)

11:10-11:40 Dopamine signalling in the bee
(Alison Mercer, Otago, New Zealand)

11:40-12:10 Modification of olfactory learning and memory induced by siRNA targeting nicotinic acetylcholine subunits in the honeybee
(Monique Gauthier, Toulouse, France)

12:10-12:30 Cellular pysiology of the honeybee brain
(Bernd Grünewald, Frankfurt, Germany)

12:30-14:00 Lunch (buffet in the lobby) and poster session

14:00-14:20 Plasticity of synaptic microcircuits in the mushroom-body calyx of the honeybee
(Wolfgang Roessler, Wuerzburg, Germany)

14:20-14:40 Neuropeptides in the bee brain
(Giovanni Galizia, Konstanz, Germany)

14:40-15:40 Session 7: Orientation and navigation
(Chair: Alison Mercer, Otago, New Zealand)

14:40-15:10 How do honeybees obtain the specific messages from dances in the darkness of the hive?
(Axel Michelsen, Odense, Denmark)

15:10-15:40 Molecular dissection of honey bee dance language: progress and prospects
(Gene Robinson, Urbana-Champaign, USA)

15:40-16:00 Break

16:00-17:00 Past, presence, future of honeybee neurobiology
Randolf Menzel

17:00-19:30 Poster session and closing of the day

Sunday, 6/13/2010

09:00-12:00 ***Session 8: Comparison with other invertebrate systems***
(Chair: Wolfgang Roessler, Würzburg, Germany)

09:00-09:30 Multi-component signals in ant communication
(Bert Hölldobler, Tempe, USA)

09:30-10:00 Neurogenetics of associative function in *Drosophila*
(Bertram Gerber, Würzburg, Germany)

10:00-10:30 Neural processing of behaviorally significant odors in the antennal lobe of the moth *Manduca sexta*
(John Hildebrand, Tucson, USA)

10:30-11:00 ***Break***

11:00-11:30 How time flies: the molecular architecture of long-term memory in *Aplysia*
(Tom Carew, Irvine, USA)

11:30-12:00 Chemosensory coding and learning in the moth *Heliothis virescens*: searching for the neuronal network involved
(Hanna Mustaparta, Trondheim, Norwegian)

12:00-12:30 ***Closing remarks***
(C Giovanni Galizia, Konstanz, Germany)

ABSTRACTS

Plenary Lecture

Martin Giurfa
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Visual cognition in honeybees: from elemental stimulus learning and discrimination to non-elemental categorization and rule extraction

Visual learning allows acquisition of new environmental information, which in turn allows adaptive responses when viewing experienced events again. This capacity is crucial in contexts such as food search, partner recognition, navigation and defense against potential enemies. It admits different levels of complexity, from the formation of a simple associative link between a visual stimulus (e.g. a specific color) and its outcome (e.g. reward or punishment) to more sophisticated performances such as categorization of objects (e.g. animal vs. non-animal) or the abstraction of rules that can be transferred to novel situations (e.g. "larger than" or "on top of"). Mastering categories and rules allows, in principle, flexible responses beyond simple forms of learning. Not surprisingly, higher-order forms of visual learning have been mainly studied in vertebrates with larger brains, while simple visual learning has been restricted to animals with small brains such as insects. Among the latter, the honeybee has paved the way for understanding the principles of elemental visual learning. Starting with the pioneering work of Randolf Menzel whose PhD Thesis characterized more than 40 years ago color learning and retention performances in free-flying honeybees, a plethora of studies have shown that bees can learn elemental associations between a variety of visual cues such as shapes, movement cues, contrasts, etc., and appetitive sucrose reward. Recently, however, the dichotomy between focusing on insects for simple learning forms, and on vertebrate for more elaborated learning forms has been broken by studies on visual cognition in honeybees, which have yielded surprising results in terms of the sophistication of the tasks that could be mastered. Here we review a spectrum of visual learning forms in bees, from color and pattern learning, visual attention, top-down image recognition, to inter-individual recognition, conditional discrimination, category learning and rule extraction. We discuss the necessity and sufficiency of simple associations to account for complex visual learning and profit from the extensive knowledge on brain organization in insects to discuss neural mechanisms underlying these visual performances.

Hiroyuki Ai

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The auditory system of the honeybee

The afferents of the Johnston's organ (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). Within these termination fields only axon terminals in the PPL, but not in DL-dSEG, are characterized by both thick processes with large varicosities and somatotopy, suggesting that vibratory signals detected by the JO are processed not only on the parallel pathway but also on the different manner in these primary sensory centers. The morphological and physiological characteristics of interneurons arborizing in these areas have been studied by intracellular recording and staining. The 77 interneurons have been identified. Twelve neurons of them have dendritic arborization in the DL-dSEG and three neurons of them have dendritic arborization in the PPL. The former are categorized into two types, the DL local interneurons and the DL output neurons projecting into the other neuropile region. DL-Int-1 is one of the DL local interneurons densely arborized in the DL-dSEG and 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. The latter 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 results show that there are at least two parallel pathways for vibration processing through the DL-dSEG and the PPL. In this study, Honeybee Standard Brain has been used as the common reference, and the morphology of three types of interneurons (DL-Int-1, DL-Int-2 and PPL-D-1) and JO afferents has been merged into the standard brain based on the boundary of several neuropiles, greatly supporting the understanding of the spatial relationship between these identified neurons and JO afferents. The visualization of the region where the JO afferents are closely appositioned to these interneurons demonstrated the difference in putative synaptic regions between the JO afferents and these interneurons in the brain. The possible roles of the parallel pathways on the vibratory processing in the primary auditory centers will be discussed.

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Chromatic and achromatic vision of bees in relation to flower patterns

By the standard of vertebrate eyes, the resolution of insect compound eye is poor. For example, the optical resolution of the honeybee eye is about 100 times coarser than our own. Therefore, in order to detect and recognize profitable flowers, bees need to use their eyes effectively. Recent behavioural studies demonstrate that the resolution of the honeybee vision is close to the limit set by optics of their eyes. On the other hand, our analysis of multispectral images of flowers shows that flower patterns have been evolutionarily adapted for optimal detection by bees. Similar to many other diurnal animals, honeybees have separate visual pathways for high spatial resolution luminance vision and for low spatial resolution chromatic vision. Such separation optimizes the signal-to-noise ratio and allows a visual system to process in parallel different aspects of images. Bees have three spectral types of photoreceptors peaking in ultraviolet, blue and green parts of the spectrum. While chromatic vision is mediated by combining the signals of all three receptors, luminance vision is mediated by the signal of the green-receptor alone. Theoretical estimates show that the honeybee eye cannot optically resolve borders of a disk subtending an angle less then 5^{O}. Results of behavioural experiments agree with this estimate - bees cannot detect circular targets subtending an angle less then 5^{O}, when the stimulus presents contrast for green-receptor (luminance pathway). Behavioural resolution of chromatic visual pathway is much poorer. Both theoretical considerations and evidence from behavioural experiments demonstrate that honeybees detect a ring having strong green-receptor contrast (bright) around a disk with weak green-receptor contrast (dim) from further distance then a dim ring around a bright disk. The distribution of contrast for blue and ultra-violet receptors does affect the ability of bees to detect stimuli, because the signals of these receptors do not contribute to the high resolution luminance vision. To learn whether flower patterns are adapted to vision bees, we recorded multispectral images of radial symmetric bee-pollinated flowers and reconstructed views of these flowers as honeybees see them from a distance. A majority of bee-pollinated flowers have a dim for the L- receptor centre and bright surround, i.e. the patterns that are easy to detect. Flowers that have not optimal for detection patterns (bright centre and dim surround) tend to be larger than flowers having dim centre and bright surround. These results suggest that patterns of bee pollinated flowers are optimised for detection by bees.

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Psychophysics of honeybee color processing in complex environments

The processing of colour information allows a visual system to detect or discriminate stimuli based on the relative distribution of spectral radiation, independent of brightness. The honeybee constitutes an interesting model to understand the very building blocks of colour vision since the neural resources available for information processing are highly constrained compared to primate vision. However, despite the relative size of the bee brain, highly trained bees that are provided with differential conditioning can learn to discriminate fine colour stimuli differences to a level that is comparable to primate vision – whilst untrained bees generalize similar colours. Why and how individual bees exhibit a large degree of neural flexibility to learn colour information remains largely unexplored, and there are exciting avenues of research to be conducted applicable to both the neuroscience and the ecological aspects of bee vision. Experiments with individual bees trained to perceptually similar colours reveals interesting insights into speed accuracy tradeoffs, and evidence of top down control in the bee brain. Evidence is presented that following differential conditioning to colour stimuli the honeybee brain forms a long term memory, but this memory is subject to decay over a period of about two weeks.

Jean-Christophe Sandoz

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Olfaction in the honeybee: multiple odour representations in the honeybee brain

Through different processing steps, olfactory systems create evolving internal representations that differently represent odors' chemical characteristics and/or biological value. In honeybees, odors are detected by sensory neurons on the antennae, which project to a primary processing centre, the antennal lobe (AL). Then two main tracts of projection neurons convey odor information to higher brain centers, the mushroom bodies (MB) and the lateral horn (LH). The honeybee olfactory system arbors two mostly non-overlapping subsystems, from the AL to the MB and LH corresponding to the lateral- and medial- tracts of projection neurons. Previous works has unraveled the representation of odor stimuli in one subpart of the AL (corresponding to the lateral tract) and in the MB, but odor representations in the medial part of the AL and in the LH have remained unaddressed. Using *in vivo* calcium imaging, we have first compared the representation of odor quality and odor quantity for a panel of aliphatic odorants in the lateral and medial parts of the AL. We find a high redundancy of the two subsystems in their response spectrum, but with some specificity in the way they encode odors' functional group and chain length information. We further addressed odor representation in the LH, which is thought to be involved in coding the biological value of odors. We used two different staining methods emphasizing respectively general activity or the responses of projection neurons, and compared the responses to floral odors and pheromones used by bees for communication within the hive. All tested odors induced clear calcium responses in the LH. Specific recordings of projection neurons showed an odor-specific map, with the same intensity but different odor-similarity relationships as in the AL. Our recordings suggest that the LH contains an odor-specific map, which may be biased for representing some odors with a pheromonal function.

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Molecular dissection of the honeybee brain: an approach to solving the mystery of 'dance communication' and the sociality of the honeybee

Aiming at identifying the molecular and neural bases of the honeybee social behaviors and the dance communication, we have conducted the ‘molecular dissention’ of the honeybee brain, in which genes that are expressed in a brain region-preferential manner, or genes whose expressions differ depending on the behavior of the honeybees were systematically searched for and identified by using the differential display method and cDNA microarray. We show here that two kinds of interneurons, termed large and small types of Kenyon cells (KCs) that comprise the honeybee mushroom bodies (MBs), a higher center in the insect brain, have distinct gene expression patterns. Based on their gene expression profiles, the large and small type-KCs are assumed to have major roles in ‘learning and memory’ and ‘division of labor of workers’, respectively. In addition, analysis of the neural activity in forager brains using a novel immediate early gene, termed *kakusei*, indicated that the small type-KCs are active in forager brains, suggesting that the small type-KCs are involved in information processing during the foraging flight. Furthermore, we identified two genes that are expressed preferentially in the ‘monopolar cellls’ in the optic lobes (OLs), which is a visual center in insect brain, and a novel gene that is expressed in a neural subpopulation located from anterior to posterior at the dorsal OL regions. These findings suggest that the advanced *functional* brain area-specification based on the differential gene expression patterns (the ‘module like-structure’) is one of the prominent features of the honeybee brain. We expect that further analysis of this ‘module-like structure’ observed in the honeybee brain would contribute to our understanding of the molecular and neural bases underlying social behaviors of not only honeybees but also higher mammals including human.

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Elucidating the path from genotype to behaviour: is epigenomics the way forward for honey bee neuroscience?

The reasons for the recent rise to prominence of the honey bee are multiple. First, the standard honey bee genome has been sequenced[1] and the brain epigenomes of both queens and workers have been determined[2]. Second, the nutritionally-controlled phenotypic polymorphism in this species offers unparalleled advantages in the study of developmental flexibility[3,4]. Third, the behaviours of queens and workers are strikingly divergent, varying from the navigational proficiency of foragers to the hive-bound omnipresent chemical influences of the queen which control many aspects of the colony's existence. Finally, since cell division is essentially absent in adult brains[5], this behavioural diversity must largely be achieved via altered synaptogenesis and the combinatorial chemistry within existing brain circuitry which are all experience dependent and are implemented together with epigenomic modifications[6].

If an understanding of the roles of epigenomic settings such as DNA methylation in development and behaviour were to be sought in *Apis*, what phases would such a project take? I will discuss some of the initial benefits and challenges for neurobiological studies in honey bees flowing from the genome-inspired projects and draw attention to the recent advances in epigenomics that are likely to reinforce the value of this organism in comparative neuroscience. The most fulfilling challenge for the bee research is to move from static data at the molecular level to that of nervous systems operating in real time and in real environments. The honeybee system is poised to allow just such a transition, from epigenomes to neural circuitry to sophisticated behaviors, all under completely natural environmental conditions.

1. Honey Bee Genome Consortium. Nature, 443, 931-949 (2006)
2. Lyko, F., Foret, S, Kucharski, R, Wolf, S, Shaefer, M. Maleszka, R. (2010) submitted
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6. Miller CA, Sweatt JD. Neuron 2007; 53: 857-869.

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Molecular insights into honeybee brain plasticity

Experience-dependent plasticity of the adult honeybee brain, in particular the mushroom bodies, has been documented in many studies. The transition between different life stages in honeybees, from nurse bees to forager bees, has been associated with increases in mushroom body size due to dendritic expansion and presumably synaptic development. Environmental factors such as enriched surroundings compared to sensory deprivation have also been shown to trigger changes in brain size in honeybees.

Here, we present first results on the molecular mechanisms underlying experience-dependent plasticity of the honeybee brain. We show that a highly conserved complex of synaptic adhesive molecules, presynaptic neurexin and its postsynaptic binding partners the neuroligins, are expressed in the honeybee brain, and that their expression increases with honeybee age corresponding to the increasing demand for sensory processing. We also show that expression of neurexin and neuroligins depends on the amount of sensory stimulation received from the immediate environment and increases significantly with learning and formation of long-term memories. This suggests that the neurexin-neuroligin-complex plays a significant functional role in honeybee brain plasticity via modulation of synaptic development and maturation in higher brain centres associated with sensory experience and learning.

Furthermore, we provide first evidence that sensory experience also triggers molecular changes in the sensory periphery, namely the expression of 7-transmembrane olfactory receptor proteins on dendrites of the antennal sensory neurons. Expression of six olfactory receptors that were shown to bind floral odorants varies significantly with changes of the scent environment the bees experience. Both the transition from hive nurse bee to outdoor foraging bee, and exposure to different flowering plants in the four seasons induced distinct variations in the olfactory receptor expression patterns. Our results suggest that similar to the molecular plasticity of higher brain centres, the molecular machinery of the sensory periphery is also plastic.

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Glutamate neurotransmission in the honeybee central nervous system

Twenty years ago, it was proposed for the first time that glutamate is a classical neurotransmitter in the central nervous system of the honeybee. Since then, the existence of a central glutamatergic neurotransmission was confirmed and we begin to understand its role in olfactory learning and memory.

Glutamatergic networks are not precisely identified. Glutamate and glutamate receptors are distributed all over the brain but variable expression levels are found within and between neuropiles. Several glutamate receptors (NMDA and metabotropic) homologous to their vertebrate counterparts are identified. In addition, glutamate-chloride channels mediating inhibitory currents are also characterised.

Several reports indicate that this neurotransmission plays an important role in olfactory memory. The modulation of glutamate neurotransmission, through an action on glutamate or by manipulating the different identified receptors, always affects early long-term memory. In addition, studies by our research group and the group of Dr. Catherine Armengaud (Toulouse, France) show that glutamate chloride channels and NMDA receptors are also implicated in other processes of learning and memory.

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The genetic and developmental evolution of social organization

Insect societies have been called superorganisms because of their complex organization for reproduction, nutrition, and defense. But how does complex social behavior evolve? What are the developmental building blocks of division and labor and specialization, the hallmarks of insect societies? Recent behavioral, genetic, and genomic studies have revealed the developmental origins in the evolution of division of labor and specialization in foraging worker honey bees, the hallmarks of complex insect societies. Selective breeding for a single social trait, the amount of surplus pollen stored in the nest (pollen hoarding) revealed a phenotypic architecture of correlated traits at multiple levels of biological organization in facultatively-sterile female worker honey bees. Genetic mapping has demonstrated that the phenotypic architecture is a consequence of a genetic architecture rich in pleiotropy and epistasis possibly affecting a reproductive signaling pathway. Gene knockdown studies and transplantation of ovaries provides strong support for the hypothesis that division of labor and foraging specialization are derived from the reproductive cycle of solitary insects and under the control of the ovaries. Ovary development in worker honey bees is under the control of a social genome that results in the joint developmental control of the immature worker larva and its nestmates that feed it.

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Molecular genetic regulation of division of labor

Together with longtime collaborators, I have discovered roles of the yolk precursor vitellogenin in honey bee behavioral traits such as nursing, foraging onset and foraging bias, and in survival traits such as oxidative stress resilience, cell-based immunity, and longevity. Some of these functions are conditional on a mutually inhibitory interaction between vitellogenin and the systemic endocrine factor juvenile hormone, which is central to insect reproduction and stress response. Many recent studies suggest that the feedback-interaction between vitellogenin and juvenile hormone is tied to nutrient-sensing insulin/insulin-like signaling genes, which can influence plasticity of honey bee behavioral physiology. I will outline our most recent understanding of these systems, and include structural information that can lead to a modern understanding of vitellogenin function.

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The social regulation of task-related plasticity in circadian rhythms in honeybees

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Honey bees switch between activities with and without circadian rhythms according to their social task. Forager bees have strong circadian rhythms, whereas nurse bees typically care for the brood around the clock with no circadian rhythms in behavior or clock gene expression. Young nurses tended brood with no circadian rhythms in behavior or clock gene expression, even under a light-dark illumination regime or when placed with brood, but no queen, in a small cage outside the hive. By contrast, nurse-age bees that were restricted to a broodless comb inside or outside the hive showed robust behavioral and molecular circadian rhythms. These findings suggest that direct interaction with the brood modulates the circadian system of honey bees. Nurse bees that were removed from the hive into individual cages in the laboratory, and were orally treated with a brood extract showed attenuated circadian rhythms in locomotor activity. This finding is consistent with the premise that the brood influence on worker activity is mediated by contact pheromones. Circadian rhythmicity is context-dependent because nurses showed circadian rhythms in locomotor activity shortly after removed from the hive, and in clock gene expression after ~16 hrs. The dynamics of rhythm development support a model positing that at least some pacemakers continue to oscillate and be entrained by the environment in nurses that are active around the clock. These cells set the phase to the clock network when the nurse is removed from the hive. These findings suggest that despite its robustness, the circadian system exhibits profound plasticity enabling adjustment to rapid changes in the social environment.

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Foraging honey bees: how foragers determine and transmit information about feeding site locations

Experiments by Karl von Frisch in the 1940's revealed that dances of successful foragers in honeybee colonies describe the locations of feeding sites they visited before, at least to human observers. One can read direction and distance of a feeder from the direction and timing of various parts of the dance: Foragers point in feeder direction with waggle runs, while shaking their bodies from side to side. This might happen directly on a flat surface with sight of the sun, or with respect to gravity inside a hive.

Experiments by v. Frisch and his students seemed to indicate that the energy expanded on the way to the feeder is used as a measure of distance ("The Energy Hypothesis"). Critical experiments in which foragers had to fly up a steep mountain slope and signaled a larger distance provided support for this idea. In this situation they had to use much more energy to reach the feeder than on level surfaces. These experiments were repeated under better controlled and less strenuous conditions. The results confirmed the suspicion that the energy hypothesis can not be correct. It is now reasonable to believe that optic flow on the way to a feeder is used as a measure of distance ("The Optic Flow Hypothesis"). Tests under various conditions strongly support this idea ("High Altitude Flights", "Tunnel Experiments"). A dancer's "optic odometer" is not affected by flight velocity. It gives different distances for equidistant feeders from the same hive, depending on the "optical" environment. “Robot Bee Experiments", intended to reveal the *nature* of bee communication, did not lead to satisfying results. The experiments possibly failed because pheromones might be used during dancing. Four chemical compounds were detected with gas chromatograph/mass spectrometry on the abdomen of foragers. They appear only on the abdomen of *dancing* foragers. The failure of robot bee experiments might also have been caused by a misunderstanding of the *nature* of waggle dances. We can show that foragers use optic flow to measure distances *in the field*, as they do under tunnel conditions, and that dances reflect the optic experiences on the way to a feeder. We suspect that recruits can visually read this optic flow by observing dancers, and use it to reach advertised feeders. Dancing foragers seem to “teach” recruits in a symbolic way to reach feeding sites. They use the same methods we apply when we train foragers to new feeders. In the following contribution it is not attempted to judge the *efficiency* of bee dances, which has been a matter of dispute, but to explain the *physiology* of this remarkable behavior.

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Olfactory information transfer during recruitment in honeybees

Honey bee colonies show several communication mechanisms to coordinate collective tasks based mainly on the transference of local information through different sensory modalities. The most studied and conspicuous is the waggle dance, which involves signal transmission and provides nest-mates with information about the location of a foraging or nest site. However, these in-hive recruitment strategies are expectable to be improved by the use of olfactory information about food sources handled by nest mates. Here we will address how the waggle dance facilitates the acquisition and the retrieval of food odor information and how prior olfactory experiences alter the interaction patterns among nest-mate within the dancing and the food-unloading context. Moreover, we considered the fact that olfactory information could affect the food preferences of foragers even if it is not acquired within the recruitment context. The discussed results show that odor learning inside the hive is an important component in the honeybee recruitment system and would have long-term consequences for foraging decisions.

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Universal laws of behavior tested in the honeybee

Two candidates for universal laws of behavior derived from work on vertebrate animals were tested in honeybees in my research. One law concerned the ubiquitous phenomenon of generalization in learning. An animal obtaining a reward for a response to one stimulus will often make that response to similar but discriminably different stimuli. Under a suitably ideal characterization, generalization gradients ought to come out exponential in shape (Shepard, 1987). In spatial generalization in honeybees, this prediction was upheld in a number of different studies. A second law concerned the weighting of different and conflicting evidence. A piece of evidence is supposed to be weighted by its recency, with more recent evidence given higher weight (Devenport et al., 1997). With the passage of time since the last evidence was obtained, overall profitability of a 'patch' rather than recency of profits should dominate. Tests with honeybees failed to uphold this law, instead finding circadian modulation of preferences, with 'patch' preference highest at the circadian time at which reward was obtained on the previous (training) day. I attempted a speculative reformulation in terms of modulation of preferences according to different oscillators.

Key words: honeybee, generalization, Temporal Weighting Rule, learning, circadian, universal law

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Tactile learning in the honey bee

During tactile learning several neural subsystems interact in the brain of the bee. Antennal movements during olfactory, tactile or gustatory stimulation lead to sensory feedbacks that in turn control three-dimensional antennal motor activity. Antennal sensorimotor feedback is the basis for the acquisition of three-dimensional tactile maps.

Sensory stimuli evoke specific antennal motor programs that optimize the sampling of sensory information. Short-term enhancement of motor activity after gustatory stimulation demonstrates that an external stimulus can modify the basic motor programs in this system. The dendritic projections of gustatory antennal sensilla into the antennal motor neuropil are the neural substrate for interactions of gustatory information with the motor system.

Motor learning is the simplest form of associative plasticity at the level of the antennal motor system. Mechanosensory information from sensilla on the antennae is associated with motor activity leading to the side-specific formation of a three-dimensional map of objects within the reach of the antennae. In contrast to sucrose dependent learning, antennal motor learning is a slow process that takes many hundred antennal contacts to develop.

Operant conditioning of antennal activity with sucrose demonstrates that the association of the salient gustatory stimulus with motor activity can induce rapid and long-lasting modifications of antennal motor patterns. Antennal motor learning, projections from gustatory sensilla to the motoneurons, and the modification of antennal activity by sucrose are the building blocks of operant antennal learning. Operant conditioning of muscle activity shows that the basic mechanisms for operant learning can be found even at the level of a single motoneuron.

Tactile PER conditioning represents the highest level of plasticity in this system. In this conditioning protocol bees learn the position of an object in the three-dimensional space around the head and they learn to discriminate form, size and surface textures. Specific antennal scanning patterns are a necessary prerequisite for discrimination of surface textures.

The antennal system of the bee is a very useful model for the study of learning because there exists detailed knowledge about neuroanatomical projections, about stimulus controlled motor output, about plasticity at different system levels, and about the different functions of sugar stimuli.

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Formation of contrasting memories in honeybees

Retrieval of an associative memory results in two contrasting memories: an extinction memory about the fact that the conditioned stimulus is no longer associated with the unconditioned stimulus, and a memory about the original CS-US association. We are studying the formation of these two memories in honeybees. Several parameters are critical for their formation, including the reward presentation during acquisition. Until now it is unclear which aspects of the reward are activated when an appetitive memory is retrieved. Therefore, we are currently investigating how the reward influences the degree of the CR during acquisition and memory retrieval.

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Molecular biology of learning and memory: from memory phases to signaling cascades

Numerous studies in species as diverse as insects, mollusks, and mammals reveal a high conservation of the molecular processes underlying learning and memory formation. In all species tested, training parameters like the number and the temporal relation between training trials critically influence the dynamic process of memory formation. The connection between training parameters and the molecular events leading to a distinct form of memory however is not well understood.

The associative olfactory learning in the honeybee that leads to a robust memory provides the unique opportunity to identify the signaling cascades triggered by conditioning. Techniques developed to monitor the activity of signaling cascades in intact honeybees made it possible to characterize the connection between training parameters and temporal modulations of signaling cascades. This analysis uncovered a dynamic network of signaling events in the antennal lobes and the mushroom bodies, which are required for the induction and/or the maintenance of 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 modify learning and memory formation internal and external parameters.

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Distributed plasticity in the honeybee brain

Early olfactory processing is fundamentally similar in animals as different as mammals and insects. Because of this similarity there have been very significant advances in understanding of sensory encoding by primary sensory cells the olfactory epitheliums of these animals. Yet a similar understanding of how sensory information is transformed at the first synapses in the brain remains elusive. The primary objectives of this research are to understand how early processing in the brain transforms sensory input, and how this transformation is modified by associative and nonassociative plasticity. The honey bee Antennal Lobe (AL) is the analog of the mammalian Olfactory Bulb (OB). The networks in the AL and OB both transform sensory inputs into spatiotemporal patterns that encode odors by a sequence of activity states, or transients, and information about the identity of the odor is in the specific sequence of states in the transient. Moreover, these networks are sensitive to modulation by feedback from other areas in the brain that represent reinforcement (e.g. food). When odors are associated, or explicitly not associated, with food these modulatory systems change the way that odors are represented by the AL and OB networks. We have shown in the honey bee, using bioimaging and electrophysiological data, that the paths that the transients take are pushed farther apart by association of odors with food reward in Pavlovian conditioning paradigms. Furthermore, nonassociative plasticity induced via repetitive unreinforced exposure to odors modifies competitive interactions between neural representations. We have also shown that disruption of this modulation by RNA interference disrupts behavioral conditioning of odors. We are integrating this information into computational models of the antennal lobes. Furthermore, this information about modulation can have far reaching impacts for understanding disease states that affect early sensory processing and for development of artificial systems for pattern recognition.

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The honey bee standard brain

Digital brain atlases serve as an platform to relate experimental data from diverse neuroscience disciplines. In insects confocal microscopy allows for integrating morphological data as 3D spatial representations in whole brains. Such digital standard insect brains are used to study genetic difference in brain structure and structural plasticity or to integrate neuronal morphologies into a common frame. The average shape atlas of honeybee, the HoneyBee Standard Brain (HSB; Brandt et al., 2005), is composed of 22 segmented brain neuropils, and is used to integrate anatomical identified neurons by focussing on sensory and central pathways. The registration of neurons into the digital space of the HSB involves the segmentation of neuropils or setting landmarks in prominent brain regions. Algorithm are used to place the neuron of interest in an appropriate space in the HSB. In order to fascilitates and optimize the integration process of anatomical data an statistical shape atlas was developed that uses algorithm for the autosegmentation of neuropil borders.

I will focus on the cellular network that underlies olfactory learning and memory processes in the honeybee. So far 50 nor so neurons and tracts are registered into the HSB. On a macroscopic level olfactory and mechanosensory networks can be compared. On a finer grain scale the atlas is used to analyse micrococircuits formed by projection neurons in the antennal lobe glomeruli and their target areas in the mushroom body calyces and lateral horn. The HSB provides a 3D framework to visualize, identify and interpret the spatial relationship of these pathways on different levels from close attachments of neighboring terminals (putative synaptic sites) to neural networks.

A further goal will be to enable researchers to use an ontology storing the data and logical connections of electrophysiological, imaging and molecular study as they relate to the anatomy of the bee brain on the cellular level.

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Dopamine signalling in the bee

In honey bees, as in humans, dopamine has many functions, including important roles in behavioural regulation and motor control. However, there remains much to learn about this amine and how it operates in the honey bee. In young worker bees dopamine signalling is affected by queen mandibular pheromone (QMP). QMP lowers brain dopamine levels, alters levels of dopamine receptor gene expression and modifies cellular responses to this amine. At a behavioural level these changes have three major consequences: aversive learning is blocked, activity levels are suppressed, and young bees are more likely to show attraction to the queen. QMP's effects on dopamine signalling provide important clues about dopamine's functions in the bee, and highlight the complexity of modulatory systems that influence the behaviour of this remarkable insect.

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Modification of olfactory learning and memory induced by siRNA targeting nicotinic acetylcholine subunits in the honeybee

Acetylcholine (ACh) is the major excitatory neurotransmitter in the central nervous system of insects and targets the numerous nicotinic acetylcholine receptors (nAChRs). The recent honeybee genome sequencing described 9 α and 2 ß nicotinic subunits that can co-assemble following multiple combinations to form several nAChR subtypes.

The 11 subunits are expressed into the brain. Patch-clamp recordings coupled to single cell RT-PCR indicated that adult Kenyon cells respond to ACh by a rapid desensitizing current and express α2, α8, and ß1 nicotinic subunits. Adult antennal lobe cells show a slow desensitizing current induced by ACh and express α2, α8, ß1 plus α7 nicotinic subunits. The role of α7 and α8 subunits in olfactory learning was studied using siRNA to induce partial silencing of their expression. Honeybees injected in the entire brain with α7 siRNA before multiple-trial olfactory learning presented poor performance during acquisition and memory tests compared to control animals. α7 siRNA injected before the retrieval tests had no effect on performance, excluding an involvement of α7 in retrieval processes. This result also indicated that olfactory perception was not depending on the presence of α7 subunit. α8 siRNA injected into the entire brain leaded to a retrieval impairment but had no effect on acquisition.

As a conclusion, we assume that nAChRs containing α8 subunit might be involved in retrieval processes whereas nAChRs containing α7 subunit might be involved in acquisition processes. As each subunit deletion induced differential effects on memory processes, both subunits could be part of two different nAChRs subtypes.

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Cellular pysiology of the honeybee brain

Ion channel gating determines the generation and propagation of action potentials and the synaptic transmission. The physiology of several honeybee central neurons was analysed using intracellular or extracellular recordings or optophysiological approaches in vivo. Several of the underlying ionic currents were characterized using patch clamp recordings in vitro. In particular mushroom body Kenyon cells as well as antennal lobe neurons are very well studied. They express voltage-sensitive Na^+ and Ca^{2+} currents that depolarize the neurons upon activation. Outward K^+ currents are rather diverse. At least four types exist: a delayed rectifier, a rapidly inactivating A-type, a slowly inactivating and a Ca^{2+}-dependent K^+ current. This diversity of K^+ channels determines the threshold and shapes of single spikes and spike trains.

Based on sequence analyses the honeybee genome contains genes coding for nine different nicotinic acetylcholine receptor α-subunits, three different GABA receptor subunits, one glutamate-chloride channel, three NMDA receptor subtypes, and two histamin-chloride channels. Physiologically characterized are currents through acetylcholine, GABA, and glutamate receptors in honeybees. The ionotropic nicotinic cholinergic receptor is probably the major excitatory receptor of the olfactory pathway. In addition to mediating fast synaptic transmission the nicotinic receptor has a high Ca^{2+}-permeability and is involved during olfactory learning. It is thus a candidate for inducing learning-dependent synaptic plasticity. GABA-induced Cl^- currents are abundant on cultured antennal lobe neurons and Kenyon cells and provide the major inhibitory system in the honeybee brain. Besides the GABAergic system, glutamate-sensitive Cl- channels provide a parallel inhibitory network within the honeybee antennal lobes. Kenyon cells express functional cation-selective AMPA-like receptors. By contrast, until now no physiological data exist on a functioning NMDA receptor on bee neurons, although its expression was shown in brain tissue.

One challenge today is to incorporate all known ionic conductances into a comprehensive cell physiological network that may explain experience-dependent neuronal plasticity. It is clear that both the nicotinic and the GABA receptors are modulated by intracellular Ca^{2+}. In addition, biogenic amines such as octopamine alter the physiology of voltage-sensitive K^+ as well as cholinergic currents. The precise interactions of the various currents and signaling cascades, however, remain to be elucidated. Nevertheless, the neurons within the honeybee olfactory pathways belong to the best cell physiologically studied insect systems.

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Plasticity of synaptic microcircuits in the mushroom-body calyx of the honeybee

The mushroom bodies (MBs) are very prominent multimodal sensory integration centers in the honeybee brain. Olfactory and visual input regions in the MB calyx are organized in relatively large synaptic complexes called microglomeruli. I will highlight recent work on structural plasticity of microglomeruli in the MB calyx associated with behavior plasticity related to polyethism and division of labor. Recent work has shown that changes in the structural organization of microglomeruli in the adult MB calyx occur at different time scales. The number and density of microglomeruli in olfactory and visual compartments are affected by brood care, behavioral maturation, sensory experience, age, and stable long-term memory formation. The different categories of plasticity in the course of an insects' life time appear to contribute differently to structural synaptic changes. Increasing evidence suggests that the resulting functional changes in MB-calyx input synapses promote long-term changes in individual behavior related to division of labor and social organisation.

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Neuropeptides in the bee brain

Neuropeptides may be the most ancient chemical messengers between neurons. As all insects, bees have are a large number of different peptides and peptide receptors, most of which have been characterized only poorly, if at all. In this chapter, we briefly review the role that neuropeptides play in insect nervous systems, and then review the specific occurrence of peptides in honeybee. A few exemplary peptide families are treated with greater detail, including among others FMRF-amid related peptides (FaRPs), SIFamid, allatostatin A (AST A). While the role of several peptides is likely to correspond to that reported for other insects, but has not yet been investigated in bees specifically (e.g. bursicon and corazonin involved in molting), a few peptides have been analyzed in honeybees (e.g. tachykinin, PBAN, sNPF, which are involved in nectar and pollen foraging). Neuropeptides are also a powerful tool for neuroanatomical studies, because they can be used to characterize small populations of neurons based on their neuropeptide expression patterns.

The talk will include a few unpublished observation about neuropeptide organization in the honeybee olfactory system, especially in the honeybee antennal lobe.

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How do honeybees obtain the specific messages from dances in the darkness of the hive?

Almost 60 years ago, Karl von Frisch interpreted the dances of honeybees, and ever since the problem how worker bees detect the dancer's movements in the darkness of the hive has been the missing link in this communication system.

Several studies have shown that worker bees may touch the dancer, and that dancers generate both substrate vibrations and local oscillatory air flows which may be detected by the workers, but the perceived information seemed to be too sloppy to allow the worker bees to calculate the location signaled in the dance. Especially, the vigorous wagging movements seemed to make it much harder for the workers to detect the median direction of the dancer.

Precise measurements of the air flows generated by mechanical models of dancing bees have revealed the existence of another component. The wing vibrations responsible for the dance sounds may also generate a spatially narrow jet air flow behind the dancer's abdomen which moves away with a velocity of up to 35 cm/s. Because of the dancer's wagging motion, workers situated behind the dancer will perceive the jet as a brief pulse of flowing air. The temporal pattern of the perceived pulses depends on the position of the worker relative to the median position of the wagging dancer.

Studies of live dancers in an observation hive have confirmed the predictions made from the laboratory studies of models, but they have also added some surprising features. The dancers are able to switch the jet on and off, apparently be changing the relative position of their wings while continuing the wing vibrations responsible for the dance sounds. In addition to the narrow jets, the dancer may generate a much broader flow of air directed behind it, thus displacing up to10 ml of air per second.

The time scale of the temporal pattern of the perceived pulses is similar to that of, for example, grasshopper songs, so it should not be too difficult for the central neurons of the workers to extract the information and calculate the median position of the wagging dancer. One may speculate that the broad flow of air may carry odor signals from the dancer.

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Molecular dissection of honey bee dance language: progress and prospects

Fascination with honey bee dance communication for almost one century has led to a wealth of knowledge on its ecological, behavioral, and sensory bases. This information provides a firm foundation for genomic analyses that aim to provide insights into the neural and molecular bases of this spectacular communication system. In this talk I discuss results of the first transcriptomic analyses of dance communication, which have been pursued according to a heuristic framework that seeks to identify modules of dance communication that can be studied more simply than the complete behavioral system. Comparative transcriptomic analyses across Apis species that differ in dance behavior have identified candidate neuromolecular systems involved in circadian, geotactic, and odometric elements of dance. These studies also have highlighted certain central nervous system regions for further study, such as the central complex and the thoracic gangion, two regions that show surprising coupling of gene expression during dance. Additional insights into odometric elements were obtained in a study that demonstrated that a small number of genes expressed in the mushroom bodies and optic lobes, including some known to be involved in learning and memory, are responsive to changes in flight distance to a feeder. I also discuss findings from octopamine and cocaine treatment studies that highlight the role of the reward system in the regulation of dance behaviour. Molecular studies are identifying neuromolecular systems that may have been the foci of change during the evolution of dance behaviour, and the consistent emerging theme is that the evolution of dance behaviour involved extensive reuse and adaptation of many existing neuromolecular systems.

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Multi-component signals in ant communication

Chemical signals mediating communication in ant societies are usually complex mixtures of substances with considerable variation in molecular composition and in relative proportions of components. Such multicomponent signals can be produced in single exocrine glands, but they can also be composed with secretions from several glands. This variation is often functional, identifying groups or specific actions on a variety of organizational levels. Chemical signals can be further combined with cues from other sensory modalities, such as vibrational or tactile stimuli. These kinds of accessory signals usually serve in modulatory communication, lowering the response threshold in the recipient for the actual releasing stimulus. Comparative studies suggest that modulatory signals evolved through ritualization from actions originally not related to the same behavioral context, and modulatory signals may further evolve to become independent releasing signals.

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Neurogenetics of associative function in *Drosophila*

Our research strives for a comprehensive account of associative learning and memory on the genetic, synaptic, cellular and behavioural level. The goal is to account for the full circuitry involved in memory formation and recall. Such research has bearings not only within the biological as well as medical sciences. Rather, it reaches out to engineering because the design of 'intelligent' technical equipment will benefit from understanding the brain; second, it reaches out to psychology as it helps to see in which sense psychological processes (e.g. memory) can be understood neurobiologically. I believe that *Drosophila* as a study case can be inspiring, as it offers a fortunate combination of modest yet sufficiently complex learning ability, simplicity in terms of cell number, and molecular similarity to rodents and man. Most importantly, the GAL4-UAS system for transgene expression allows for research strategies with remarkable analytical power. In my lecture, I want to give some recent examples of such research.

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Neural processing of behaviorally significant odors in the antennal lobes of the moth *Manduca sexta*

We investigate odor-modulated behavior and neural processing of odor mixtures in an experimentally favorable model system, *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 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 led to recent analysis of odor-dependent interactions with host plants. 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 (olfactory) lobe of the brain. Mounting evidence points to coincident 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 olfactory-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. [Supported by NIH grant DC002751]

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How time flies: the molecular architecture of long-term memory in *Aplysia*

Memories can last from a few seconds to a lifetime. In my talk I will provide an overview of variety of experimental approaches that we employ in my laboratory to study the neuronal basis of these diverse forms of memory, focusing especially on long-term memory. We use the marine mollusk *Aplysia* to explore these questions because its nervous system affords significant advantages for identifying synaptic, biophysical, and molecular changes underlying different stages of memory. The fundamental goal of our experiments is to achieve an understanding of the mechanisms by which the nervous system acquires, stores, and retrieves information across a wide range of temporal domains.

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Chemosensory coding and learning in the moth *Heliothis virescens*: searching for the neuronal network involved

We are studying chemosensory coding and learning in heliothine moths, a subfamily of numerous species distributed on all continents, including some of the most devastating insects in agriculture. Because of their well developed pheromone communication with interspecific interactions, their intimate interactions with plants based on olfactory and taste cues, as well as their ability of learning and memory, they are interesting models in studies of chemosensation. In the present study we focus on how biologically relevant plant odour- and taste information is handled by neurons in the central nervous system of *Heliothis virescens*, searching for the neuronal network underlying chemosensory coding and learning. In previous studies we have functionally characterised olfactory receptor neurons for plant odorants according to primary and secondary odorants, using electrophysiological recordings from single olfactory receptor neurons (ORNs) linked to gas chromatography and mass spectrometry. So far, biologically relevant odorants for 20 ORN types have been identified. The gustatory receptor neurons (GRNs) have been classified by screening putative tastants, resulting in classification of four major GRN types. On this background we are studying how the information from the receptor neurons is mediated/ processed in the central nervous system of this moth. By intracellular recordings from neurons in the antennal lobe, the lateral protocerebrum and the suboesophageal ganglion (SOG), combined with fluorescent staining for confocal microscopy followed by 3-D reconstructions, the neurons are physiologically and morphologically characterised. The central olfactory neurons are characterised according to their responses to single primary odorants and mixtures; some showing responses exclusively when tested for mixtures. The central gustatory neurons integrate information about several taste modalities from GRNs on all appendages. After reconstructions, the olfactory and gustatory neurons are integrated into the recently made standard brain atlas of *H. virescens* (Kvello et al. 2009). The second version of the atlas with integrated antennal lobe glomeruli further allows identification of the glomeruli innervated by antennal lobe neurons in addition to their projections in the Calyces of the Mushroom Bodies and in the lateral protocerebrum (Løfaldli et al. 2010). Whereas most central gustatory neurons are located in the SOG/tritocerebrum, some of them project in several areas of the brain, including the lateral protocerebrum, anterioventral of the olfactory area. Ongoing studies focus on neurons in the lateral protocerebrum, input as well as output neurons, including possible integration of olfactory and gustatory information.

POSTER PRESENTATIONS ABSTRACTS

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Mechanisms and purpose of dual-pathway odor coding in the honeybee

In the olfactory system of the honeybee *Apis mellifera*, uniglomerular projection neurons relay sensory information from olfactory sensory neurons to higher brain regions via two distinct pathways, the lateral and the medial antenno-cerebral tract (l- and m-ACT). Neurons within these two tracts exhibit complementary coding strategies regarding concentration dependence, breadth of odor tuning and mixture representation. We used a rate-code network model to describe how these distinct coding properties can be brought about by varying the strength of lateral inhibition and gain control. We were able to reproduce l-ACT specific odor representations using strong lateral inhibition and gain control, while m-ACT specific coding properties were reproduced using weak lateral inhibition and no gain control. Our results suggest that the neuronal networks which these pathways are part of have different network parameters and are at least partly segregated. In addition, our findings indicate that the development of two parallel systems with complementary coding strategies may reflect an evolutionary adaptation to cope with the search/approach task that honeybees face during foraging in their natural environment.

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The what and where of operant learning in *Drosophila*

In the last couple of years, B.Brembs showed that world learning (the process assigning value to sensory stimuli) and self learning (the process assigning value to a specific action or movement) were two components of operant learning. The two elements depend on different molecular substrates and they interact: world learning inhibits the formation of self learning. My project is to decipher the molecular bases of self learning using the genetic tools available in Drosophila in combination with behavioral read-outs. We know that an inhibitor of PKC prevent self learning formation. My goal is to identify which PKC is involved in this behavior and where it is involved in the fly brain.

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Development of an experimental setup for electrophysiological characterization of decision making via operant conditioning in a virtual environment in the honeybee

We are going to present an experimental setup that enables us to record from bees extracellularly during walking on an air-floated styrofoam ball. The walking trace of the behaving bee is detected by optical computer mice and can be recorded and visualized with software developed in our lab. The bee will soon be able to control a virtual reality in a way that for the bee's perspective the natural relation of own movements and environment-movements is achieved. Therefore we developed a cone-shaped screen on which a beamer projects the virtual environment. So far we are able to hold on stable extracellular recordings in the PE-1 region of the mushroom body for a few days. Our goal is to combine long-term recordings with an operant conditioning paradigm to study behavioral decision making on the network level.

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Localization of phosphorylated CREB in the mushroom bodies of honeybees

The transcription factor CREB (cAMP response element binding protein) plays an essential role in the formation of long term memory (LTM). CREB is required for the induction of a consolidated LTM by inducing gene expression after becoming phosphorylated (pCREB) by several kinases. A huge body of work analyzed this CREB phosphorylation after learning but little is known about its role in honeybees (*Apis mellifera*), a well known invertebrate model system for learning and memory. Our aim is to analyze the role of *Apis mellifera* CREB (AmCREB) in the formation of LTM in the honeybee. The AmCREB gene has been thoroughly analyzed and several splice variants have been characterized.

In my project I aim towards localizing phosphorylated AmCREB (pAmCREB) in learning associated neuropils. I visualize pAmCREB in fixed bee brains using an antibody against the phosphorylated form of the vertebrate CREB which also detects phosphorylated AmCREB. pCREB immunoreactivity can be observed in a wide range of cell nuclei including nuclei of neurons known to be involved in processing sensory information. Furthermore, pCREB staining can be detected in specific regions of the mushroom body calyx and peduncle. Ongoing experiments focus on changes of pAmCREB in bee brains after appetitive conditioning by analyzing pAmCREB-immunoreactivity.

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Odour coding in projection neurons of *Apis mellifera* changes under olfactory adaptation: A Calcium Imaging Study

Projection neurons (PNs) connect the insect antennal lobe (AL) with the mushroom body (MB) and lateral horn. Odours are coded in PN by their glomerulus specific input patterns. We recorded the calcium activity in the postsynaptic dendrites of the l-ACT (lateral antennocerebral tract) PNs in the frontal glomeruli of AL of the honey bee Apis mellifera after backfilling these PNs with the calcium sensor dye Fura-2 via their axons. The antennae were stimulated with 8 different pure odorants before and during exposure of the bee to a constant air flow extracted from a functional bee colony. We have asked whether the odour coding on a relative scale changes in the course of such a biologically relevant adaptation. We found that colony air leads to a global excitation in all of the frontal glomeruli and changes their responses to the test odours. Glomeruli number 47, 35, 42 and 43 become more inhibited whereas number 17, 33 and 36 showed more or less an equal distribution of excitatory and inhibitory responses to the tested odours. In general colony air leads to a reduction of glomerular responses to the odours but interestingly it has also caused response enhancement for some combination of the glomeruli and odours. This indicates that a strong background stimulus such as the colony air can have different forms of excitatory and inhibitory effects over the activity of different glomerular networks. Overall, we found a higher degree of similarity in the response patterns between glomeruli and odours within an animal than between the animals. We conclude that the changes induced by adaptation to hive air will lead to perceptual changes possibly by enhancing particular forms of odour discrimination.

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Proteasome mediated protein degradation modulates long term formation in the honeybee

Proteasomes are multi-protein complexes that are degrading proteins. To be recognized by the proteasomes, their target proteins are tagged with ubiquitin by an ubiquitin ligase before degradation. The ubiquitin-proteasome system plays a crucial role in a number of neuronal processes including axon guidance, synaptic development, synaptic function and synaptic plasticity. Moreover, a role of the ubiquitin-proteasome system in long-term memory formation has been demonstrated. However, these results remain contradictory. In the crab Chasmagnathus the ubiquitin-proteasome system is necessary for the formation of long-term memory, whereas activity of the ubiquitin-proteasome system in the amygdala of rats restricts formation of a long-term memory in a fear-conditioning paradigm.

Here, we examine the role of the ubiquitin-proteasome system in the honeybee (*Apis mellifera*), an invertebrate model system for investigating learning and memory formation. We used an appetitive pavlovian learning paradigm, the olfactory conditioning of the proboscis extension response (PER). We systemically injected two proteasome-inhibitors at different time points before and after acquisition and tested their effects on learning and memory formation. We demonstrate that long-term memory, but not middle-term memory, is enhanced when these inhibitors are applied after acquisition. This enhancement depends on the number of training trials and on the inter-trial interval (ITI). We conclude, that proteasome activity restricts long-term memory formation depending on the inter-trial interval and the number of trainings trials. We hypothesize that this restrictive process is important to prevent inadequate strong memory formation maintaining an animals ability to learn about changing situations and environments.

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Sleep in honeybees: Sleep deprivation impairs the consolidation of navigational memory

Sleep seems to be ubiquitous within the animal kingdom. In vertebrates various studies have shown the importance of sleep for consolidation of long-term memory. Sleep is less well characterized in invertebrates but has been found in most investigated species. In some cases, like Drosophila and the honeybee (Hussaini et al. 2009) correlations between sleep and memory consolidation have been found.

We studied sleep behavior in honeybees in the context of memory consolidation in natural conditions. To test if sleep and memory consolidation are correlated, we compared the duration of foraging and the sleep inside the hive of individually RFID-marked bees either trained to a nearby feeder or foraging naturally. In addition we monitored return times and sleep behavior after translocating RFID-marked bees to unexpected release sites. We sleep deprived bees by shaking them over night and analyzed their sleep behavior after sleep deprivation and compared their navigational memory with bees that spend a normal night in their hive.

We found that sleeping time is independent of the difficulty of the navigational tasks. Sleep deprivation leads to a changed structure of sleep after the sleep deprivation. Sleep deprivation does also not change foraging performance at a previously trained feeder, but impairs the consolidation of newly acquired navigational memory.

These findings suggest that undisturbed sleep is important for the consolidation of new navigation memory in honeybees.

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Mushroom body output neurons encode odor-reward associations

Neural correlates of learning and memory formation have been reported at different stages of the olfactory pathway in both vertebrates and invertebrates. However, the contribution of different neurons to the formation of a memory trace is little understood. Mushroom bodies (MBs) in the insect brain are higher-order structures involved in integration of olfactory, visual, and mechano-sensory information and in memory formation. Here we focus on the ensemble activity of single MB extrinsic neurons (ENs) which were recorded extracellularly when honeybees learned to associate an odor with reward. ENs provide output from the MB, a higher order neuropile known to be involved in sensory integration of several modalities and in memory formation. In unconditioned animals, ENs displayed broad odor response spectra showing responses to a large fraction of tested odors. A large group of ENs (~40%) changed their odor response spectra in the course of differential odor conditioning by loosing or gaining odor sensitivity for specific odors. We found that this response switching was dominated by the rewarded conditioned stimulus (CS+) which evoked exclusively recruitment. The remaining ENs did not change their qualitative odor spectrum but modulated their response strength quantitatively. Modulation was again dominated by increased response strength to the CS+. About 140 ms after stimulus onset both types of units represented the CS+ most differently from the CS- and control odors. The learned response (proboscis extension) appeared about 330 ms later. It is concluded that the population activity of MB output neurons predict the meaning of the stimulus (reward) and may provide the prerequisite for the expression of the learned responses.

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Response adaptation can explain temporally sparse code in the insect Mushroom body

Population of Kenyon cells in insect brain Mushroom body respond to the olfactory stimuli with only a brief activity. This sparsening is a progressive feature of Mushroom body circuitry. Here, we investigate the role of inhibition in temporal sparseness of the input to Mushroom body. We show with imaging techniques in the presence of pharmacological GABA blockers the activity time course remains unchanged while observing ensemble activity in stained Kenyon cells in the calyxes of Honeybee. Therefore, we suggest an alternative and possibly parallel hypotheses based on the known neural intrinsic properties such as spike frequency adaptation and short term synaptic depression to explain the observed data. Both spike frequency adaptation and short term synaptic depression differentiate the signal in way that their sequential effect provides the system the desirable sparse representation. We study this hypotheses with a simulation of simplified path way of olfactory circuits and show the experimental result on time course of activity ensemble of Kenyon cells can be explained by suggested neuron intrinsic attributes.

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Projection neurons from the same glomerulus are synchronized

Glomeruli of olfactory systems are commonly regarded as functional units. However, in the honeybee as in most model species there are multiple output neurons originating from each glomerulus. Thus, the convergence of functionally equivalent receptor neurons onto projection neurons is incomplete. Does the incomplete convergence result in differing response characteristics among projection neurons from the same glomerulus? We elucidated odor-evoked and spontaneous activity simultaneously in up to 12 neurons innervating two adjacent glomeruli at high temporal resolution. A 2-photon laser scanning microscope (2PLSM) was constructed specifically for imaging of honeybee antennal lobe projection neurons stained with the calcium-sensor FURA2 dextrane. Anatomical image stacks with sufficient resolution for reconstructions of the cells were acquired in vivo. Using a correlation-based measure of synchrony, it could be shown that projection neurons that are housed within the same glomerulus are indeed synchronized to a high degree, during odor-evoked activity as well as during rest. Determining synchrony was done separately for three signaling states: signals of excitation, signals of inhibition, and rest. Careful analysis of synchrony across signaling states revealed that, strikingly, synchrony of signals of excitation is even higher during spontaneous signals as compared to signals that occurred during odor stimuli. Therefore, receptor neurons are unlikely the cause of within-glomerulus synchrony.

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A brief history of RoboBee

RoboBee is a joint project of the groups of AI (Prof. Rojas) and neurobiology (Prof. Menzel) aiming for the precise reproduction of the honeybee dance communication system.
Motion trajectories of waggle dances were analyzed and used to formulate a realistic dance model. Using small cameras, our robot is the first biomimetic honeybee robot that can see and react on its environment.

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Do bees use gradient map-like structures for orientation and navigation?

A basic navigation strategy bees are using is path integration[1], extracting the rotatory component from the sun compass and the translatory component from their visual odometer[2]. It is known since the famous experiments by von Frisch and Lindauer that bees relate the vector information of their flights also to extended landmarks[3]. In 2003, L. Jacobs and F. Schenk presented a model for the organization of map-like representations in spatial memory[4]. They suggested that such a representation could consist of two parts: the bearing map, a kind of gradient (long ranging landmarks) based map and the sketch map, a view-based map which stores the relational positions of landmarks as seen from a vantage point. We try to test this concept by tracking bees with the harmonic radar. Different groups of bees were tested which performed their orientation flights in different landscapes. We find that bees generalize between landscapes according to the similarity of gradients (e. g. tree rows, irrigation canals or contrasts between areas of grass land). Our findings indicate that bees may use a kind of a sketch map.

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Flower color evolution of *Papaver rhoeas* (Papaveraceae)

Ever since Lotmar (1933) measured *Papaver rhoeas*' spectral reflection, the flowers of this species have been indicated as an example of a red UV reflecting flower. A report by Dafni et al (1990), however, suggested that populations of *P. rhoeas* might differ with respect to their reflectance in the UV range. This difference results intriguing considering that it would have a direct impact in the way pollinators perceive these flowers.

In order to evaluate the potential differences in the chromatic appearance within *P. rhoeas*, different populations of this species around the Mediterranean region were evaluated with respect to their spectral reflectance properties. Additionally, in an attempt to test the hypothesis that *P. rhoeas* may have originated from the East Mediterranean and in order to evaluate the possible ancestral condition of *P. rhoeas*' flower color, we measured the spectral reflectance of *P. humile*, *P. umbonatum* and *P. carmeli*, species pointed out as *P. rhoeas*' closest relatives and distributed along the East Mediterranean border. The spectral data collected supports the idea that populations of *P. rhoeas* differ with respect to their spectral properties and suggest that the ancestral condition within *P. rhoeas* is the UV absorbing flower condition.

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Honeybees integrate learned and communicated flight vectors in navigation

Honeybees share their knowledge about a feeding place with their nest mates. By means of a ritualized behavior, the waggle dance, they communicate locations by encoding vector information. Since many of the recruited bees will have visited another feeding place before they will have to decide between two distinct destinations: either the communicated vector or the flight vector they have learned before. We carried out an experiment in which we tracked the flight of recruits with harmonic radar. We asked whether recruits compare these two vectors and whether they decide between them. Recruits indeed choose between the two vectors. If the directional components of the two vectors are more similar (30° rather than 60° difference) the learned vector is more frequently used. Decision for the communicated vector requires more information (more waggle runs are followed). In the 30° condition some bees also perform novel shortcut flights between the communicated and learned location in both directions, indicating that bees integrate communicated and experienced information in a common spatial representation.

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Neuronal representations of multimodal associative learning in mushroom body extrinsic neurons of the honeybee (*Apis mellifera*)

The mushroom body (MB) in the honeybee brain plays a crucial role in sensory integration and memory formation. In order to elucidate the role of MB extrinsic neurons in multimodal associative learning and memory formation, we performed extracellular long-term recordings of single neurons in the output region of the MB, while the bee performed a classical conditioning task. We asked, whether single neurons respond to either olfactory and /or visual stimuli, and whether they exhibit learning-dependent plasticity.

First, we presented a set of odors and colors before training to get the baseline neuronal activity. Then, we conditioned the bees differentially to colors and combinations of colors and odors, and tested all stimuli seperately and pairwise 30 minutes after training.

For response detection, we compared the ISI distributions before and short after stimulation in all test phases.

Significant responses (Wilcoxon rank-sum test, $p<0.05$) were analyzed by calculating the trial-averaged firing rate of each unit in the different test phases.

Two physiologically different groups of units were identified. One responded initially before training towards either one or both modalities and reduced their responses after learning, probably reflecting the memory trace for the association formed after training.

The second group was recruited after learning and responded either to the rewarded stimuli specifically or to all stimuli, rather encoding context cues, present in reward learning.

These results indicate that MB extrinsic neurons show a range of learning related plasticity and may play a key role in sensory integration during reward learning.

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The effect of protein synthesis inhibitor on reversal learning in the Honeybee

In classical conditioning, animals learn to associate an originally neutral stimulus (CS) with a biologically significant stimulus (US).In the honeybees *Apis mellifera*, forward pairing of an odor and reinforcement elicits the proboscis extension reflex. Honeybees are also capable of differential learning, where they learn to respond to a reinforced odor (CS+) and not to respond to a nonreinforced odor (CS-). Following Pavlov (1927) CS+ leads to an excitatory association, CS- to an inhibitory association, and the balance of the response strength expresses the balance between excitatory and inhibitory associations. In reversal learning, the contingencies of the conditioned stimuli are reversed, and honeybees learn to adjust their response to the new rule. This study investigated the effect of protein synthesis inhibitor (emetine) on the memory formed after reversal learning using two groups of honeybees: summer bees and winter bees. Blocking protein synthesis in summer bees inhibits consolidation of the excitatory learning whereas the consolidation of the inhibitory learning was blocked in the winter bees.

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Learning to navigate: orientation flights of young honeybees

While young honeybees perform orientation flights they inspect the hive and the nearby environment. During these flights they learn everything they require to return safely from their foraging flights which they perform after they have completed their orientation phase.
To find out what exactly is learned during these orientation flights we used the harmonic radar technique which enables us to track the complete flight path of bees. We recorded the very first orientation flight and displaced the bees afterwards to a known and an unknown area and vice versa. With this experiment we tested the hypothesis that bees find their way back to the hive faster when displaced to a known rather than to an unknown area. This would demonstrate that bees learn about the spatial relations of extended and local landmarks from their very first orientation flight onwards.

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Honeybees increase the use of waggle dance information when using private information becomes unrewarding

Social insect foragers often have access to both social and private information about the locations of food sources. In honey bees (Apis mellifera), foragers can follow waggle dances (social information) to obtain vector information about the location of profitable food sources or they can use route memories (private information) acquired during previous foraging trips. The value of vector information is poorly understood and currently debated. Theory and vertebrate examples predict that social information should be favoured when using private information has a low benefit. To test this we trained foragers to a high quality sucrose feeder which subsequently became unrewarding. Our results show that as the use of private information became unrewarding, the trained foragers increased the time spent following waggle dances advertising an alternative food source with the same odour. A significant proportion of foragers successfully switched to the food source indicated by dances (13.5% after 1 day, 25% after 2 days). However, the trained foragers also showed a strong attachment to the known but currently unrewarding feeder (4.9 visits in 4 hours over 2 days following the cessation of reward), even after repeatedly following dances advertising a profitable alternative (11.6 dances). Successful recruits to the food source advertised by the waggle dances had more social information about the novel food source, i.e. they followed dances for longer (4.45 vs. 3.19 waggle runs per dance). Our data suggest that honey bee foragers follow a strategy that is quite conservative in terms of switching from one food patch to another, presumably because carbohydrate food sources have a high chance of becoming rewarding again after periods of being unrewarding.

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Field versus Lab: how sucrose acceptance-thresholds in the field affect complex behaviours of honey bees in the field and laboratory

Laboratory studies in honey bees have shown correlations between sucrose responsiveness and complex behaviours like division of labour, learning and memory formation. This study analyses the significance of sucrose perception for behaviour under free-flying conditions and describes the importance of the behavioural context under natural conditions for the interpretation of responses in the laboratory. In a first set of experiments we determined the acceptance for different sucrose concentrations of free-flying bees in the field and compared it with their proboscis extension response (PER)-thresholds in the laboratory. The data demonstrated that it is not possible to infer from the PER in the laboratory which concentrations the respective bees were accepting in the field. In a second set of experiments intrinsic factors that modulate PER-thresholds were analysed in individual foragers. The experiments demonstrated a very fast modulation of PER-thresholds after drinking sucrose solution from a feeder only for a few seconds. The behavioural context in the field has thus a strong influence on the behavioural responses in the lab. In a third set of experiments we analysed the learning performance and 24h retention in the field and laboratory in bees with high and low sucrose acceptance-thresholds. In both conditions bees with low sucrose acceptance-thresholds showed significantly faster and better acquisition and 24h retention than bees with high thresholds. PER-thresholds of these two groups of bees did not differ significantly. The results show that foraging behaviour in the field is a good predictor for learning behaviour in the field and in the laboratory.

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Visual Navigation: the role of different landmark cues for homing honeybees

Honeybees use memorized visual representations to find back to their hive and to profitable food sources. Prominent landmarks can play an important role in pinpointing the exact goal location. Especially the final approach seems to be mediated by comparing the current retinal input with a stored retinotopic representation of the visual scene around the goal.
In this study we explore the role of different landmark cues and their relevance for honeybee navigation. In an indoor flight-arena we used high-speed cameras to record honeybees approaching a previously learnt food source located between three landmarks. We then introduced unpredictable variations of the landmark arrangement between returns and analyzed the effects on the 3D-flight trajectory and the bee's body and head orientation. We find that under all tested conditions stabilizing head movements lead to a behavioural elimination of rotational components from the optical flow pattern, which facilitates depth perception from motion parallax.
Changes in the spatial landmark configuration affect the overall flight pattern and search duration. The effects are small when the three landmarks remain in place and their texture is changed all at once. But can honeybees make use of a landmark's texture, if it labels one landmark? Honeybees rely stronger on pattern cues than on the spatial configuration of landmarks if the pattern labels the landmark close to the food source and if the pattern is dissimilar from the surrounding visual scene.

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The altruistic infertility of honeybees: a physiological approach

The success of a honeybee colony depends largely on the reproductive altruism of the worker bees, which usually remain sterile, and instead help their mother queen to reproduce. As yet, little is known about the genomic basis of this spectacular form of altruism. In this study, 16 sterile and 16 reproductive workers of two colonies were differentially analyzed with microarrays, using whole bodies. 1284 genes were found to be differentially expressed, of which 871 up-regulated in fertile workers and 413 up-regulated in sterile workers. GO-enrichment analysis demonstrated that more than 25% of the GO-terms enriched in reproductive workers are linked to mitosis, meiosis or oogenesis. But interestingly, the enrichment of GO-terms involved in muscle contraction, metabolism, flight behavior etc. in sterile bees indicates that 18-day old sterile workers are clearly foraging, whereas the fertile workers of the same age are not. This is in accord with theoretical predictions that reproductive workers should tend to carry out less work and less risky tasks inside the colony. Particular interesting genes are odorant receptor 156, which has a nearly 2-fold up-regulation in sterile workers, and farnesyl pyrophosphate synthase (juvenile hormone synthesis), odorant binding protein 9 and 7, chemosensory protein 5 which were up-regulated in fertile bees. Several of these candidate genes lie within the region of previously identified QTLs linked to differences in worker reproductive capacity in honeybees. These clues point at physiological pathways starting with chemosensory sensitivity modifying both reproductive and foraging behavior. Overall, our results provide unprecedented insight into the detailed physiology of non-reproductive honeybee workers.

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Honeybee serotonin receptors and their involvement in phototactic behavior

By screening the honeybee genome, we found three candidate genes encoding for putative serotonin (5-hydroxytryptamine, 5-HT) receptors. We used this sequence information to isolate the corresponding receptor cDNAs. Comparison of the deduced amino acid sequences with sequences of characterized 5-HT receptors of other species suggested that one receptor belongs to the 5-HT1 (Am5-HT1) and the other two receptors to the 5-HT2 receptor class (Am5-HT2α and Am5-HT2β).

Am5-HT1 was pharmacologically characterized in stably expressing HEK293 cells. Adenylyl cyclase activity was attenuated by the activation of Am5-HT1 by 5-HT. The agonists 5 methoxytryptamine (5-MT) and 5-carboxamidotryptamine were able to imitate this effect, whereas methiothepin, prazosine and WAY100635 were able to block 5-HT effect either completely or the partially.

An anti-Am5-HT1 antiserum detected a protein with a molecular mass of 50 kDa in western blot analyses. This antiserum was used to investigate the Am5-HT1 expression pattern in immunohistochemical experiments. Strong Am5-HT1 immunofluorescence was observed in the ocellar nerves, in the three optic ganglia and in the α- and β-lobes, the pedunculi, the lip and the basal ring of the mushroom bodies.

Finally, behavioral experiments suggest an important role of the Am5-HT1 receptor in phototactic behavior. Feeding of 5-HT to worker honeybees results in a decrease of phototactic behavior. This 5-HT action could be mimicked by feeding the Am5-HT1 agonist 5-CT. In contrast, the Am5-HT1 antagonist prazosine prevents the 5-HT-induced decrease in phototaxis.

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Associative odor reward learning modifies odor representations in the honeybee antennal lobe

We investigated the effect of associative learning on early sensory processing, by combining classical conditioning with *in vivo* calcium imaging of secondary olfactory neurons, the projection neurons in the honeybee antennal lobe. We observed changes in the global response strength and the spatial pattern of odor representations after appetitive odor conditioning. After conditioning the global response strength to the rewarded odor (CS+) was greater than to an unrewarded odor (CS-), and the representation of the CS- became less prominent in the spatial response pattern of the CS+/CS- mixture. The change in spatial response pattern can be traced back to glomerulus specific learning effects, depended on the glomerular response profile in the untrained bees. The data is consistent with a neural network model of the antennal lobe, which we based on two plastic synapse types and two well known memory forming rules: (1) associative, reinforcer dependent plasticity at synapses between olfactory receptor terminals and projection neurons, and (2) Hebbian, reinforcer-independent LTP and LTD-like plasticity at feedback synapses from local neurons to receptor neuron terminals. The observed changes strengthen the idea that odor learning optimizes odor representations and facilitates the detection and discrimination of learned odors.

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A six second olfactory trace in the honeybee

Our understanding of the neuronal mechanisms of classical conditioning, where a neutral stimulus (CS) is associated with a temporally overlapping reinforcing stimulus (US), has made substantial progress in recent years. However, when the CS and US are temporally disjunct, a non-associative trace of the CS is necessary to build up a transient "bridge" allowing the nervous system to form an association with the US. Little is known about the properties of such stimulus traces. We therefore characterized odor traces and the formation of trace memories by studying appetitive olfactory trace conditioning in honeybees. In trace conditioning the US is presented after the CS has terminated, but the CS is nonetheless associated with the US and learned to be predictive. We found that odor traces maximally last between 6 and 10 s. Associative odor memories formed during trace conditioning did not differ in odor specificity or odor generalization profile from odor memories formed during conditioning with overlapping CS and US. Trace conditioning already worked after a single CS-US pairing, showing that odor traces were formed already during the first CS presentation. The initial part of an odor stimulus was transformed into the odor trace, whereas the late phase of a persistent odor stimulus did not influence the trace memory. Odor traces were robust against the interference by other odors.

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Previous olfactory experience influences olfactory generalization in honey bees and humans

Generalization and discrimination of stimuli determine the perceptual space of both animals and humans. Both are needed to effectively distinguish and integrate environmental signals.
Both honey bees and humans show remarkable similarities in their olfactory processing and both species need to either discriminate or generalize between olfactory stimuli. Therefore we analyzed the impact of learning and perceptual history on generalization in *Apis mellifera* and *Homo sapiens*.
Honey bees as well as humans were trained on two odours (honeybees: 1-hexanol, 2-octanol; humans: methylhexanoate, ethylpentanoate) which were presented at different frequency ratios (25/75, 50/50, 75/25 in honey bees; 75/25 and 25/75 in humans) in several training trials. We conditioned the honey bees in a differential appetitive Pavlovian paradigm (CS+/CS-) and recorded their proboscis extension reflex (PER). In humans, we conducted a double-blind experiment. We presented the two odours as “odour 1” and “odour 2” to the participants prior to the experiment. During training and test, the participants had to rate the odours and categorize the binary mixtures in a forced choice as either odour 1 or odour 2.
We measured the degree of generalization by recording the response to a novel odour (the binary mixture of 1-hexanol and 2-octanol and the additional odour 2-nonanol in honey bees; the binary mixture of methylhexanoate and ethylpentanoate in humans) after the training in both species. Both species were tested during the training or with a short delay, respectively, and after 24 hrs.
Our findings show that the frequency ratio with which an odour occurred during training had an impact on the degree of generalization of other odours. In both species, the novel odour was more frequently generalized toward the odour that had occurred less often during the training trials. Possibly, the higher incidence of training/reward trials has led to a more distinct neural template for that odour, moving the novel odour away from it.
This sensory learning effect disappeared after one day, while the appetitive conditioning memory was stable for this time period, suggesting two distinct neural mechanisms.

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Olfactory processing in honeybee Kenyon cells and the involvement of the GABAergic system

I investigated the representation and processing of odor information in mushroom body (MB) clawed Kenyon cells (KC) of the honeybee *Apis mellifera*.

KCs receive olfactory input in the lip neuropil of the MB calyces. They are the second order interneurons in the olfactory pathway and exist in especially high number compared to the MB afferent and efferent neurons. This suggests a reorganization of the coding principles.

It was revealed in several studies in honeybees and other insects that individual cells respond in a sparse manner upon olfactory stimuli, i.e. each cell responds only to selected odors and its responses consist of only few spikes.

I analyzed the odor concentration dependency and - selectivity of KC responses. I could (1) show that individual KCs are not selective for narrow concentration ranges and (2) the response magnitude over the monitored region is positively correlated to odor concentration; (3) responses after odor offset also show concentration dependency.

Sparseness may be a result of general or specific inhibition provoked by the neurotransmitter GABA. Application of GABA receptor antagonists revealed that responses to high odor concentrations are mediated by GABAergic inhibition via ionotrophic and metabotrophic GABA receptors. This must be understood as a gain control mechanism.

I applied a calcium imaging technique using Fura-2 as calcium-sensor combined with odor stimulation to analyse the KC activity in the MB-calyces *in vivo*.

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Comparison between Selegiline and SIM-2 in neural protection

Aim: The aim of this study was to compare the neuroprotective effect of selegiline and SIM-2.

Material and methods: The left sciatic nerves of new born rats were axotomized in the middle of thigh, then they were devided into 3 groups depended on the type of treatment : 1_treated with Selegiline 2- treated with SIM-2 3_ injected normal saline as control.Injections were continued for 21 days. After this period they were sacricified by transcardiac perfusion and Spinal cord was prepared for microscopic study. Total motoneurons counts were done in anterior horn of spinal cord and motoneurons reduction were calculated. Apoptosis in these neuron were studied by ultrastructure technique.

Results: Motoneurons reduction were decreased in the group which were treated with SIM-2 and increased in the control group. The ultrastructural study of mitocondria showes changes known as cromatolysis.

Conclusion: Neuroprotective effect of SIM-2 is more than Selegiline and farther more studies is necessary to recognize this.

Key words: Selegiline-Sciatic nerve axotomy-Apoptosis.

How to find the…..